Acta Botanica Neerlandica **15** (1966) 419–433

TRANSPORT, IMMOBILIZATION AND LOCALIZATION OF NAPHTHYLACETIC ACID-1-^{14}C IN COLEUS EXPLANTS

H. VEEN

(Centre for Plant Physiological Research, Wageningen, The Netherlands)

(received October 15th, 1965)

SUMMARY

The transport and metabolism of naphthylacetic acid -1-^{14}C in explants of *Coleus* was studied by means of the liquid scintillation counting technique in combination with thin layer chromatography.

Microautoradiography was used to study the intracellular localization of the auxin.

The gross radioactivity in the tissue is mainly fixed just below the donor block. In this part of the explant the radioactivity is present in the cytoplasm next to the cell wall. Although all the radioactivity in the receiver block represents the auxin itself, tissue extracts showed an intense metabolic turnover of the auxin. A study was made of the time-dependency of the production of several compounds and the ratio between the compounds in extracts of different parts of the explant. Finally, the chemical identity of the substances is discussed.

INTRODUCTION

The growth substances known to control the vegetative growth of a wide variety of higher plants generally have to be translocated from their site of production to their site of action. The importance of auxin translocation is obvious in such phenomena as apical dominance and abscission.

Studies on abscission have been carried out either with whole plants, or explants (plants: VENDRIG, 1959; JACOBS and co-workers, 1964; explants: ADDICOTT and co-workers, 1949; LEOPOLD and co-workers, 1964; GARDNER and COOPER, 1943; LUCKWILL, 1956; GORTER, 1957 and 1964). Explants of bean and of *Coleus* have been most widely used. Those of *Coleus* are particularly well suited for studying the relationship between the abscission of the petioles and the transport of auxin.

JACOBS (1962) emphasised, the translocation of the auxin in the tissue must be known in detail before we possibly can understand the results of investigations on the abscission of petioles of explants.

A previous study which revealed some characteristics of the transport of auxin in explants of *Coleus*, has been published elsewhere (GORTER and VEEN, 1966). It was stated in that paper that almost all radioactivity remained in the tissue when the auxin (carboxyl-labelled naphthylacetic acid), was applied to the apical proximal end of the explants. More detailed information on the immobilization and the localization of auxin in the tissue of explants of *Coleus* will be given in the present paper.

Material and method

The material used has been described in detail by Gorter (1964). All material originated from a single clone of *Coleus rhenaltianus*, vegetatively propagated throughout the years. The plants were grown in the greenhouse.

The explants comprised a node with 5 mm pieces of stem above and below and two petiole stumps of 5 mm each (see Fig. 1). They were taken from the younger part of the plant where the apical proximal internode was about 10 mm long. These plants consisted of five internodes.

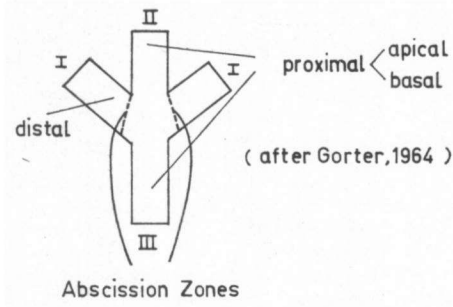

Fig. 1. Scheme of an explant used in the experiments

The explants were placed horizontally in petri dishes on small foam plastic cushions. Each petri dish contained 5 explants. The dishes remained in the dark at 20° C.

The growth substance was applied in agar blocks (2 per cent) of 0.02 ml volume each placed on the apical or basal ends of the stem parts (position II and III resp.).

The α-naphthylacetic acid -1-^{14}C (NAA, carboxyl labelled) was obtained from The Radiochemical Centre, Amersham, England, and had a specific activity of 5.28 mc/mmole (28.4 μc/mg). Afterwards a new batch with a specific activity of 8.27 mc/mmole (44.5 μc/mg) was used. The compound appeared to be stable and showed no any radiochemical impurities as checked by thin layer chromatography with different solvent systems. The final concentration of the auxin in the agar donor blocks will be given together with the experiments.

Radioactive assay was done by the liquid scintillation counting technique. Agar blocks were transferred into small vials and equilibrated with 2 ml distilled water for 24 hours to permit diffusion of the radioactive substance from the blocks into the water. 1 ml of ethylalcohol was then added to the vials. The scintillation solution contained per litre:

800 ml dioxan
160 ml ethyleneglycolmonoethylether
48 g naphthalene
9.6 g 2.5-diphenyloxazole (PPO)
0.48 g 1.4-bis-2-(5-phenyloxazolyl)-benzene (POPOP)

The radioactivity in each vial was determined in a Packard Tri-Carb liquid scintillation spectrometer. Samples were counted for at least 10 minutes at 0° C. The counting efficiency in these experiments is 51 per cent for an aqueous standard of ^{14}C in the 100–1000 window at a high voltage setting of 1040 V. The background in our experiments was usually 45 cpm. All counting data were checked statistically on $P < 0.05$. Under these conditions 1 cpm corresponds to appr. 1.5×10^{-10} mmole NAA.

To measure the degree of quenching the counts are recorded in two separate channels. The ratio of these two channels provides a fast correlation with counting efficiencies determined with the aid of an internal standard. Quench correction is carried out by this channel ratio method, so that the addition of an internal standard to all vials is unnecessary.

The identity of the radioactive substances in the agar blocks as well as in the tissue was determined by thin layer chromatography. The blocks and tissue parts were frozen in dry ice and afterwards extracted for several hours with hot acetonitrile. The liquid was evaporated to dryness and the residue was taken up in 0.5 ml of acetonitrile. Extraction with acetonitrile was preferred to alcohol because it had been found by BACH (1961) that it gave almost quantitative extraction of radioactivity while it minimizes the extraction of other cell constituents. Moreover, acetonitrile is a free-radical quenching solvent, it is miscible with water, evaporates easily and is relatively non-toxic to plants (STOWE, 1963). The different samples were spotted on chromatograms of silica gel G and developed with isopropanol/ammonia 25 per cent/water (8:1:1). The chromatograms were covered with a "Melinex" polyester film (thickness 6.25 μ) to avoid chemical reactions on the film plate. A Kodak medical X-ray no screen film was then placed against the chromatogram ("sandwich"); the film was exposed for at least one week. After developing the films, places on the chromatogram that had given blackening on the film were marked and the silica gel from each area was carefully scraped off the glass and transferred to a Tri-Carb counting vial. The scrapings were suspended in 2 ml of distilled water and 1 ml of ethanol for several hours and regularly shaken to elute the organic material into the water. To this suspension was added the scintillation liquid. The radioactivity found at different spots on the chromatogram are expressed as a percentage of the total radioactivity extracted.

The total amount of the acetonitrile-soluble ^{14}C in the tissue pieces was measured by taking a sample of 0.1 ml of the final 0.5 ml extract. This sample was counted in the usual way.

The radioactivity, remaining in the tissue pieces after acetonitrile extraction was determined by grinding the tissue in a mortar in 2 ml of distilled water and 1 ml of ethanol. The final suspension was counted in the spectrometer in the usual way. The radioactivity extracted at the end of 5 hours by the water-ethanol mixture was 10–20 per cent of the amount of radioactivity extracted with acetonitrile. The chemical nature of this rest-radioactivity was not further investigated.

The use of a relatively polar solvent scintillation system such as dioxane-water prevents the adsorption of radioactive polar material on silica particles and thus losses by self-absorption (SNYDER, 1964). Like Snyder we found no counting loss caused by any substantial self-absorption in an aqueous sample of ^{14}C in the presence of silica particles, as is illustrated by the next data. A 10 μl sample of an aqueous standard (0.0528 μc/10 μl) was spotted on a thin layer of silica gel. Without chromatographing, the spot was scraped off and counted in the spectrometer. In this case we were able to recover 95.4 per cent of the radioactivity of the original sample. We then measured the recovery of chromatographed spots of the same sample. The ratio between the sum of all radioactivity present in the scrapings of a chromatogram and an aqueous sample directly pipetted into a counting vial provides us a check of the utility of the whole thin layer chromatography technique. The mean value of the recovery from four chromatograms was 91.3 per cent. From these data we concluded that thin layer chromatography in combination with liquid scintillation counting is an useful tool in the separation and quantitative determination of compounds labelled with ^{14}C.

The localization of the auxin at cellular level was studied by microautoradiography. Minute pieces of *Coleus* tissue were frozen in isopentane cooled with liquid nitrogen (— 175° C). The pieces were dehydrated at — 80° C with anh. acetone for 8 days. The acetone solution was refreshed every day. The acetone was replaced by a monomer metacrylate mixture to which was added a polymerisation initiator. This mixture was kept at a temperature of 2° C. Finally the small tissue pieces were placed in gelatine capsules filled with a metacrylate mixture. Heating at 60° C then starts the polymerisation. (For details see GIELINK, SAUER and RINGOET, 1966).

Sections 5 to 10 μ thick were cut on a rotating microtome. The slices were collected on glass slides, and a piece of stripping film (Kodak AR 10) was placed on the slides. An exposure time of 54 days usually gave the best results in this particular case. After this period the films were developed. Slices were studied by means of a Zeiss photomicroscope.

RESULTS

Some general data on auxin transport in *Coleus* explants have already been published (GORTER and VEEN, 1966). The results will be shortly summarized here:

By applying donor blocks (with auxin) and receiver blocks (plain agar blocks) in various positions, it was possible to make a simultaneous study of basipetal and acropetal transport in these explants. NAA translocation in *Coleus* tissue is mainly basipolar, but there is also some acropetal movement. The radioactivity found in the receivers at the basal proximal end (position III) after 10 hours of application of auxin at the apical proximal end (position III) is about 20 times that found in the receivers placed on the distal cut surfaces (position I).

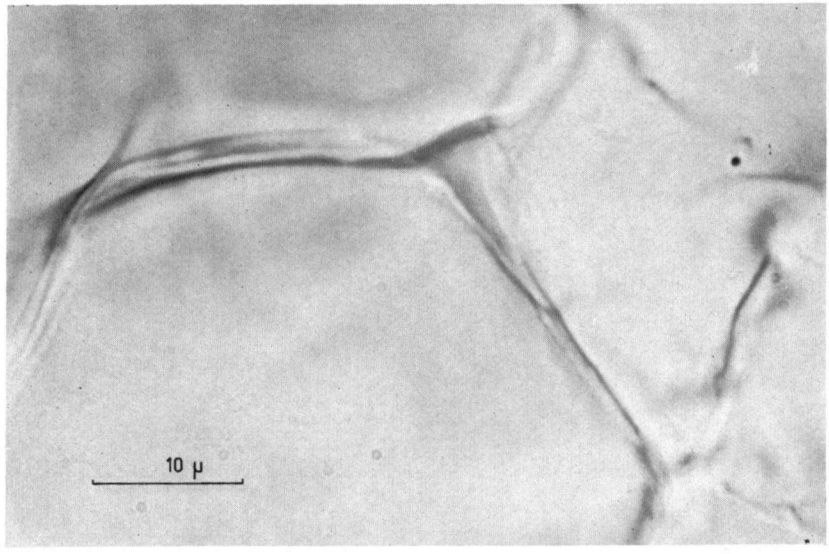

Fig. 2. Photograph focussed on the tissue.

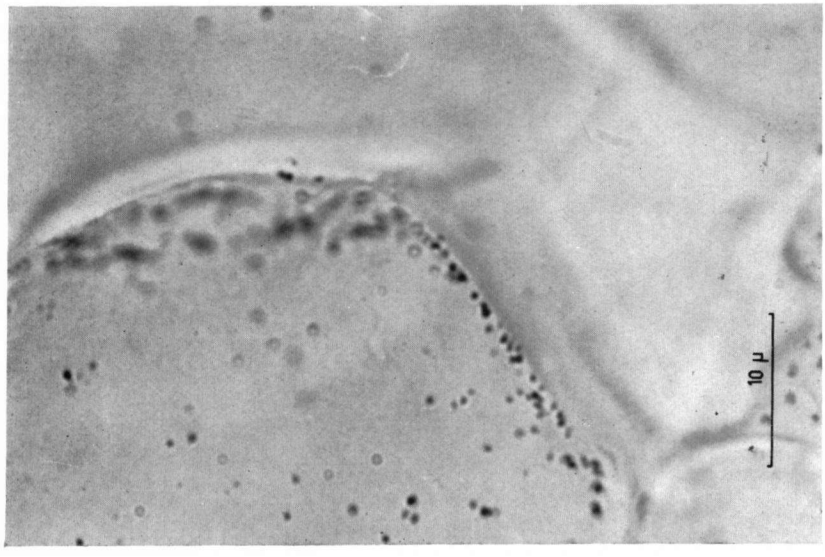

Fig. 3. Photograph focussed on the stripping film covering the slices

Figs. 2 and 3. Photographs of microautoradiograms showing radio-activity inside parenchymatic pith tissue of *Coleus* stem sections.

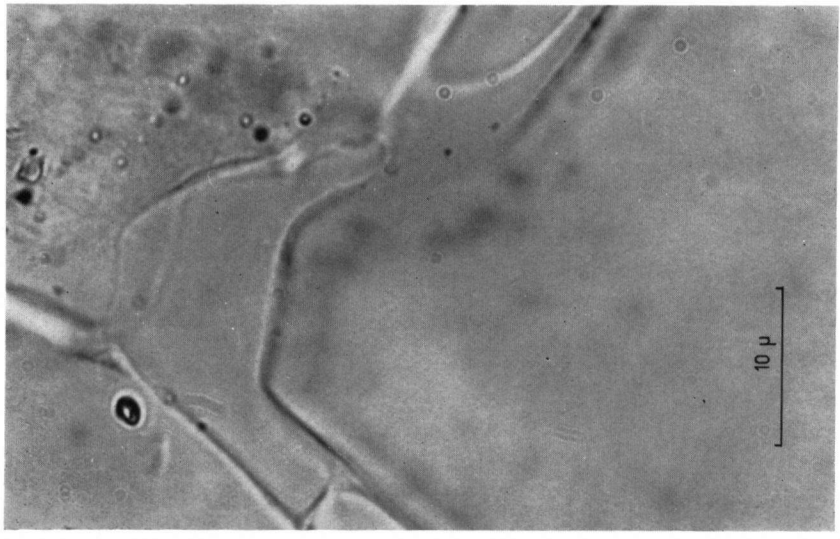

Fig. 4. Photograph focussed on the tissue.

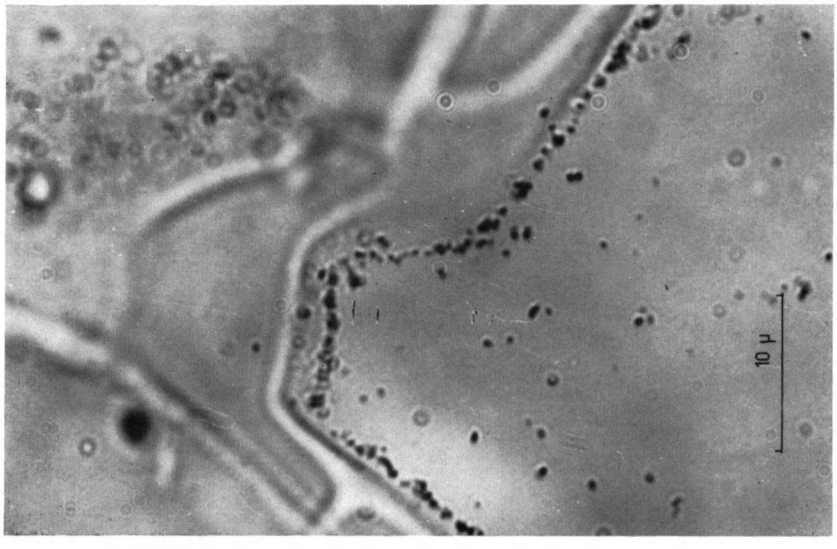

Fig. 5. Photograph focussed on the stripping film covering the slices.

Figs. 4 and 5. Photographs of microautoradiograms showing radio-activity inside parenchymatic pith tissue of *Coleus* stem sections.

PLATE II

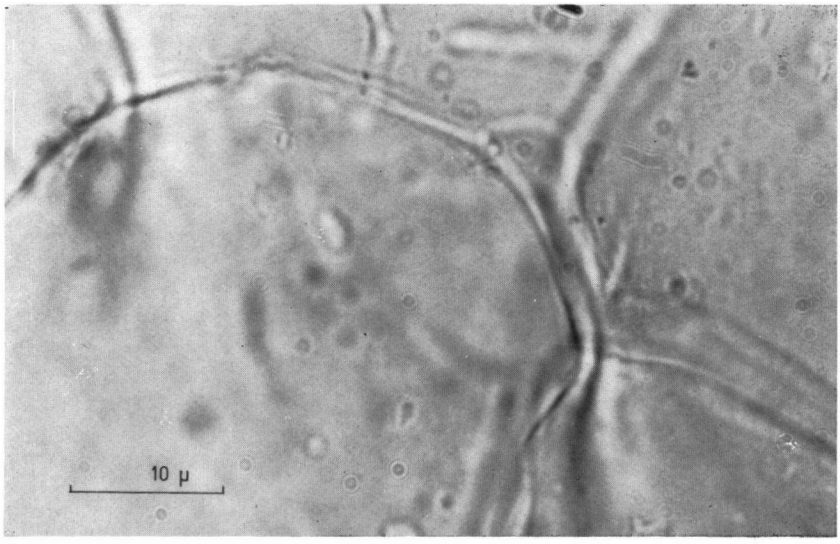

Fig. 6. Photograph focussed on the tissue; it shows plasmolysis.

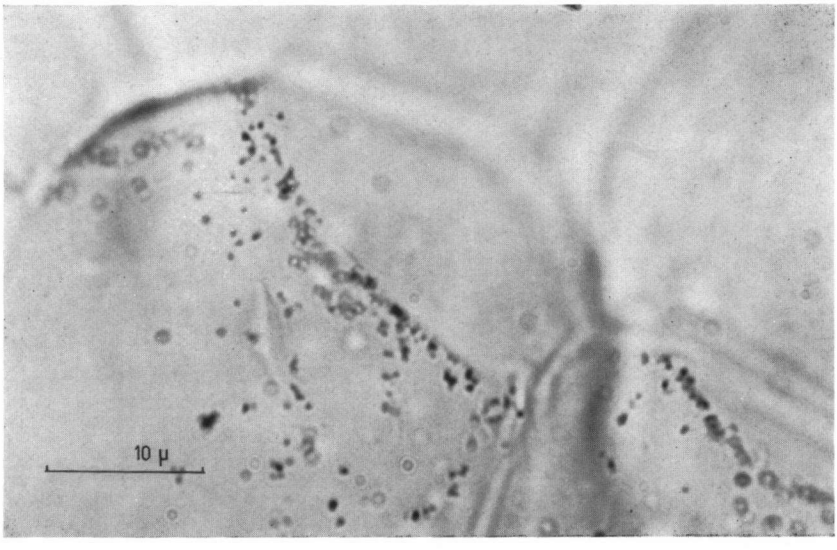

Fig. 7. Photograph focussed on the stripping film covering the slices; it shows plasmolysis.

Figs. 6 and 7. Photographs of microautoradiograms showing radio-activity inside parenchymatic pith tissue of *Coleus* stem sections.

PLATE III

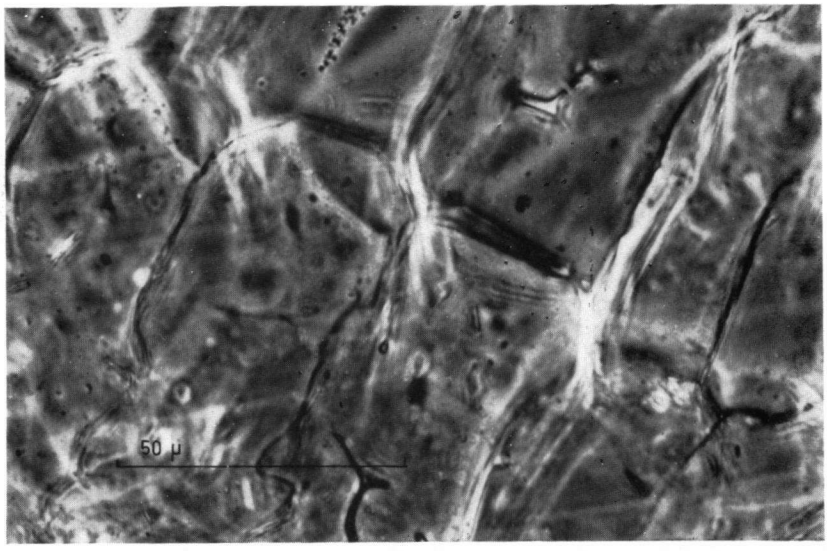

Fig. 8. Photograph focussed on the tissue, and made with phase contrast microscopy; it shows plasmolysis.

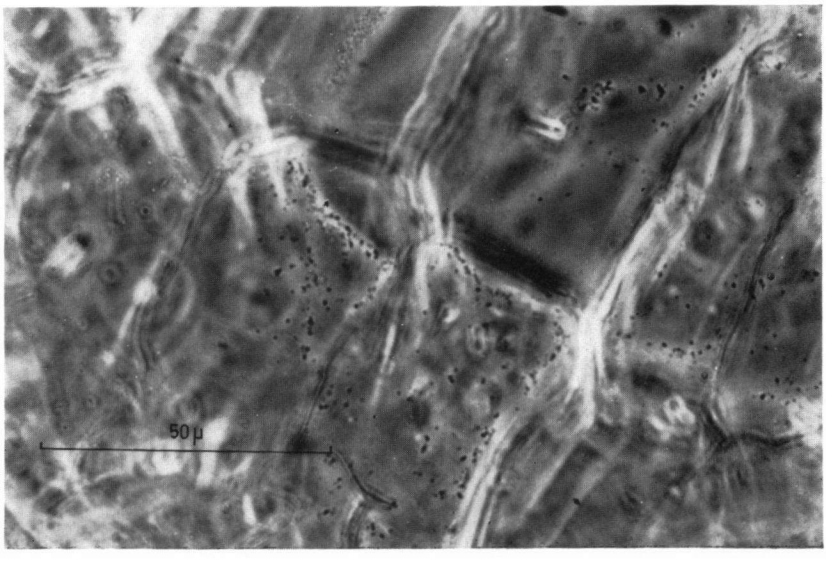

Fig. 9. Photograph focussed on the stripping film covering the slices, and made with phase contrast microscopy; it shows plasmolysis.

PLATE IV

Increase of radioactivity in basal receivers is linear with time during the first 10 hours; after 10 hours a maximum is reached, after which the transport comes to a standstill. The activity in the basal receivers after 24 hours is only a very small percentage of that lost from donor blocks (viz. 1.4 per cent). Nearly all radioactivity is immobilized in the tissue.

From preliminary experiments we obtained good evidence that the decarboxylation rate of NAA by *Coleus* tissue can be neglected. This was studied by a manometric method described by YARDLEY (1964). In the first 6 hours the amount of free $^{14}CO_2$ in the atmosphere is less than 0.1 per cent of the initial concentration. We therefore concluded that nearly all radioactivity which entered the tissue is fixed in the explant. To study the gross distribution of the radioactivity in the explant we carried out the following experiment. Donor blocks (conc. 2.4×10^{-4} M) were applied to the apical proximal end of the explant. After 24, 48, 72 and 96 hours the explants were divided into different parts:

part 1 = apical proximal part
part 2 = petiole stumps
part 3 = the node
part 4 = basal proximal part.

These parts were extracted and the radioactivity counted as described earlier. The data are given in Table 1.

From the data of Table 1 we conclude that about three quarters of the total gross radioactivity fixed in the explant is retained in the 5 mm apical proximal part below the donor block.

The intracellular localization of the radioactivity in this part of the explant was further studied by microautoradiography. Hitherto reports have been published of two methods of localising growth substances at the cellular level. The first was applied by THIMANN and BETH (1959). They removed the potential site of action from cells to which auxin was applied afterwards. This was done with *Acetabularia* from which the nucleus had been removed. A clear effect

TABLE 1

Gross distribution of acetonitrile-soluble ^{14}C after auxin application at the apical proximal part of the explant. Donor Conc. = 2.4×10^{-4} M. Extractions after 24, 48, 72 and 96 hours. Explants were divided in Part 1: apical proximal part, part 2: petiole stumps, part 3: the node, part 4: basal proximal part. Results are given as cpm per mg dry weight and as percentages of the total extracted radioactivity of a whole explant.

Part	Time in hours							
	24		48		72		96	
	cpm	%	cpm	%	cpm	%	cpm	%
1	9881	69.7	17135	72.8	21207	81.0	16061	75.8
2	382	1.8	111	0.3	71	0.2	97	0.3
3	744	12.0	1002	9.0	1026	8.3	1338	11.6
4	1130	16.4	2197	17.8	1370	10.5	1639	12.3

of auxin was found even on the enucleated cells. The second method was applied by GALSTON and KAUR (1959). Auxins labelled with radioactive isotopes were applied to cells which were fractionated by centrifugal forces in an attempt to determine into which part of the cell the auxin had found its way. The conclusion to be drawn from such studies is that auxin does not bind itself to any of the cell particles and that its site of action may be in the nonparticulate phase of the cytoplasm (GALSTON and PURVES, 1960).

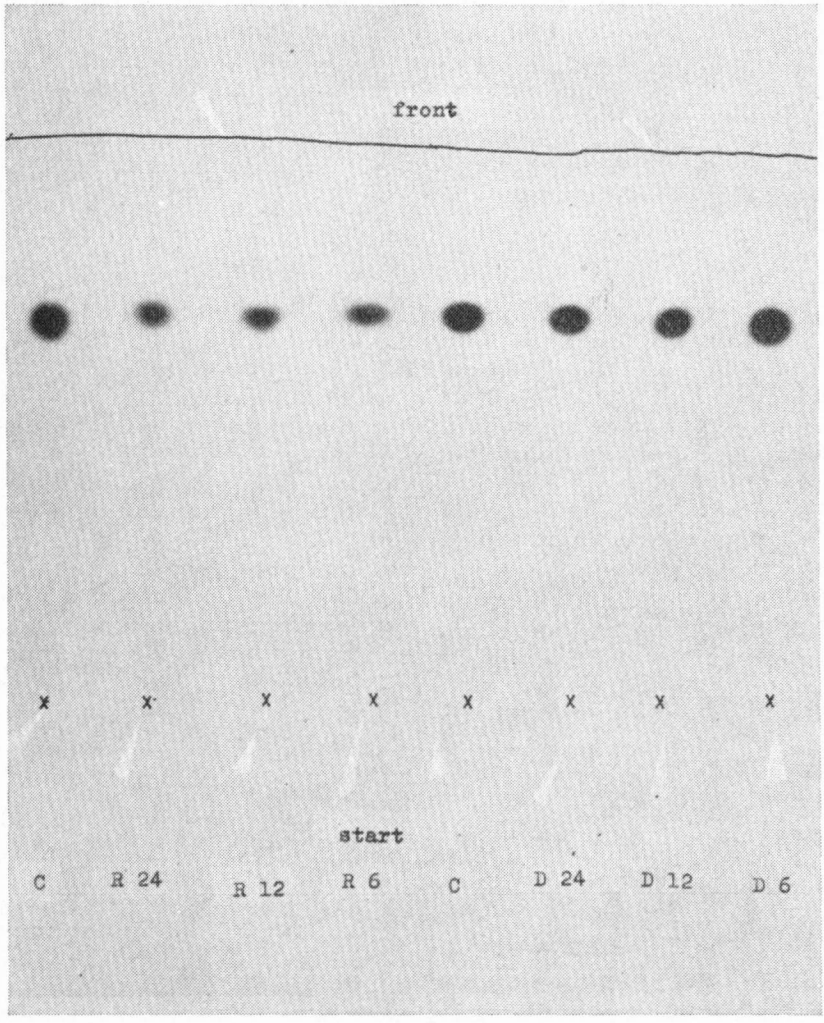

Fig. 10. Photograph of a Kodak no screen X-ray film, which had covered a thin layer chromatogram for about a week. After 6, 12 or 24 hours only one compound can be recovered in receiver and donor blocks with a *Rf*-value, which is the same as NAA-^{14}C (c).

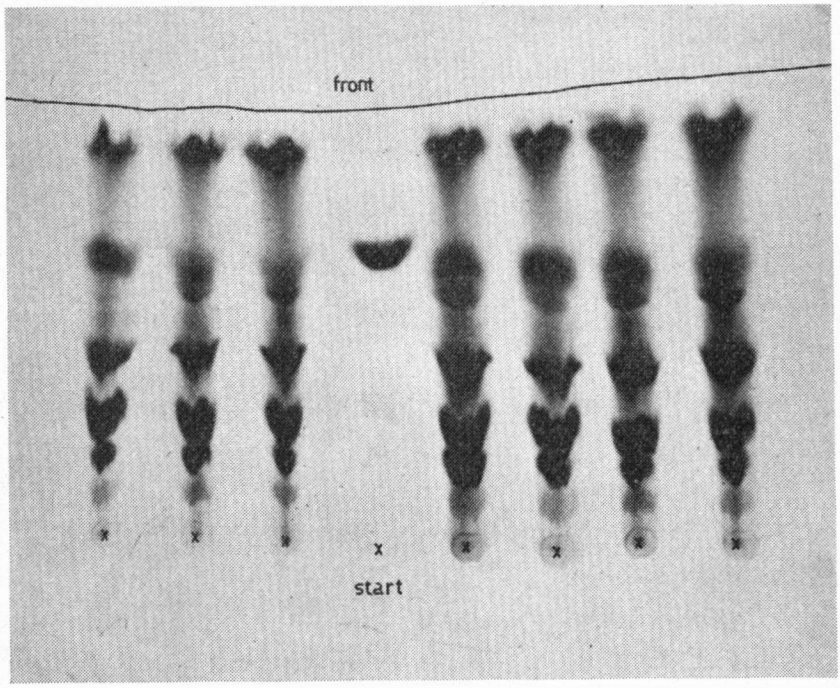

Fig. 11. Photograph of a Kodak no screen X-ray film, which had covered a thin layer chromatogram for about a week. NAA is converted into at least six other compounds. All the spots were of identical extracts made after 24 hours.

There is also clear evidence from many other experiments that auxins are attached to some receptor entity in the cell, although it is possible that the labelled auxin is so weakly attached that the link is soon broken by centrifugation. For this reason it seems necessary to locate the site of action by other methods, e.g. microautoradiography. Therefore the next experiment was carried out. NAA-^{14}C was applied to the cut end of the apical proximal part of explants of *Coleus*. The donor concentration was about 4×10^{-4} M NAA. After 24 hours the apical proximal part of the explant (in which the bulk of the radioactivity is fixed) is divided in small parts of appr. 1 mm and treated further for autoradiography as described earlier.

These microautoradiograms clearly demonstrate the presence of radioactivity in the cytoplasm next to the cell wall, as is shown in the Figs. 2–9. As a rule two photographs were taken of each object, the first focussed on the tissue, the second on the film. In one case some plasmolysis could be observed; here the blackening of the film exactly followed the cytoplasm. It should be remembered that these photographs were taken from longitudinal slices of the central pith of the apical proximal part of a *Coleus* explant. Cross-sections of the same tissue produced a more intense blackening per unit of area above the cortical tissue than above the pith tissue. But in the cortical part

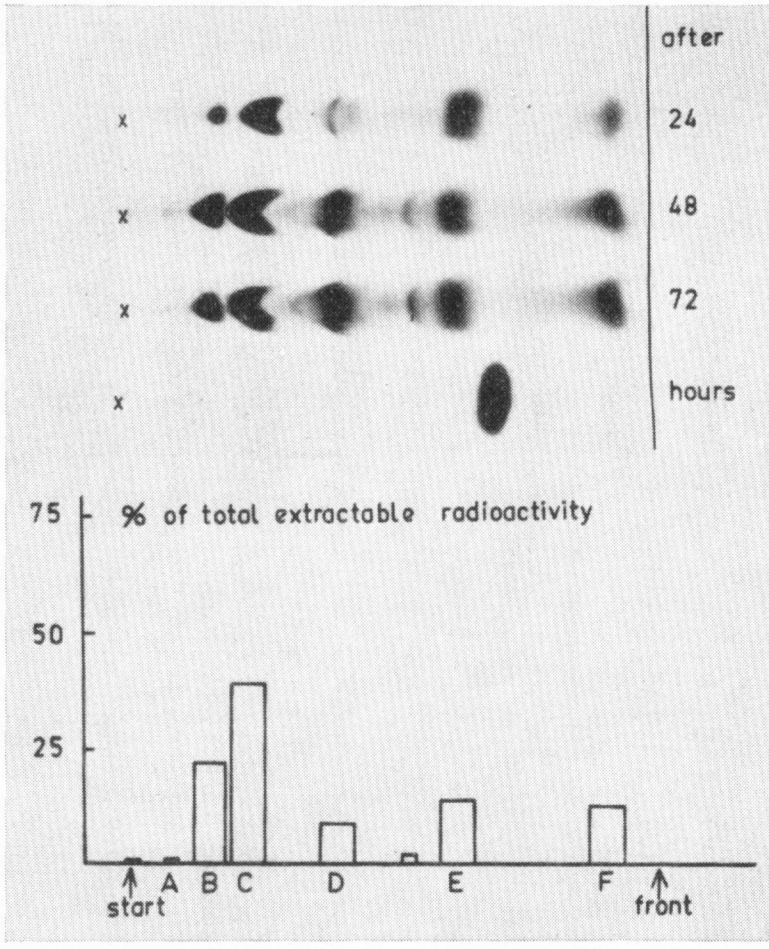

Fig. 12. The radioactivity at different spots on the chromatograms, expressed as a percentage of the total extracted ^{14}C material. The upper part of the figure shows a film which covered the thin layer chromatogram for about a week, the lower part gives the radioactivity found at each spot expressed as a percentage of the total amount of radioactivity extracted by acetonitrile. Only the data of the 24-hour experiment are plotted.

of the sections, the intracellular localization of the radioactivity was not so distinct.

JACOBS (1965) recently showed that for IAA and 2, 4, — D the transport velocity was the same in both pith tissue and "cortical tissue" (which included the vascular tissue). For both auxins more net loss was observed into cortical tissue than into pith tissue. Jacobs explains this by the difference in cell number; the cortical tissue contains ten times as many cells as the pith tissue, although the areas are identical. The difference between the blackening of the

parts of the film which were above the cortical and the pith tissue, as found in our own experiments, can be explained in the same way.

According to previous published evidence (GORTER and VEEN, 1966), only NAA could be detected in receiver blocks after 6, 12 and 24 hours (see Fig. 10). But chromatography of an extract of a whole explant some hours after application of the auxin already showed a conversion into several compounds (see Fig. 11).

The radioactivity found at different spots on the chromatogram is expressed as the percentage of the total acetonitrile-soluble ^{14}C-material. This is shown in Fig. 12, the upper part of the figure being an example of an autoradiograph from chromatograms obtained by placing a film on the thin layer chromatogram and the lower part showing the radioactivity found at each spot of the chromatogram expressed as the percentage of the total amount of radioactivity extracted by acetonitrile. In this particular case only the data of the 24 hours experiment are plotted.

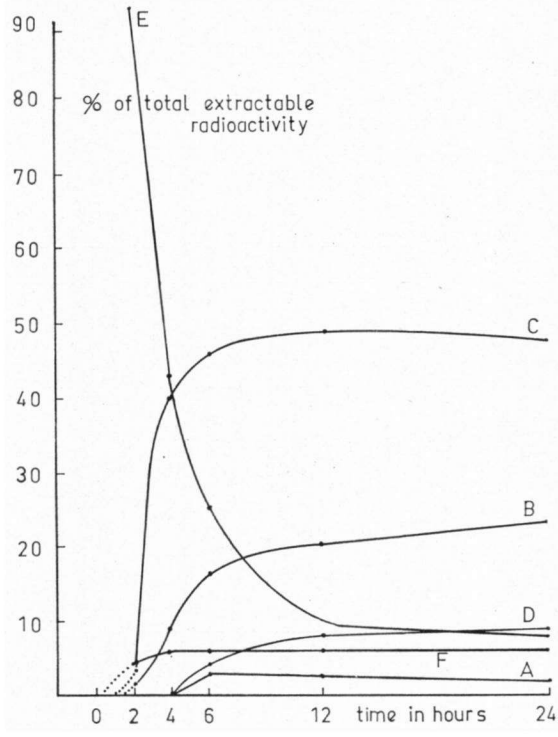

Fig. 13. The amounts of the various compounds in tissue extracts, expressed as a percentage of the total extracted radioactivity, in relation to the time.

The relative amounts of the various compounds (cf. Fig. 11) were determined at different times after application of the auxin at the apical end of the explants. Donor conc. was 4×10^{-4} M. After 2, 4,

6, 12 and 24 hours the donor blocks were removed and the whole explant was extracted. The extracts were chromatographically analysed in the usual way the results being shown by Fig. 13. A striking feature is the rapid decrease of the extractable amount of compound E. After 6 hours only 25 per cent of the original amount

TABLE 2

Gross distribution of acetonitrile-soluble ^{14}C and the relative amounts of some metabolites in two different regions of an explant. Apical proximal part means the apical stem end of the explant just below the donor block. Donor Conc. 2×10^{-4} M. The basal proximal part means the basal stem end of the explant at a distance of 25 mm from the donor block. Extractions were made after 24, 48, 72 and 96 hours. The gross distribution is given as cpm per mg dry weight. The amounts of the different compounds are given as the percentages of the total extracted radioactivity present in apical and basal parts resp.

	Gross distr.	Comp. E probably NAA	Comp. C probably NAAsp	Comp. F probably NAAglu
Apical prox. part				
time	cpm	%	%	%
24	12043	10.7	53.2	11.5
48	17554	5.8	48.3	10.2
72	18376	6.0	39.4	13.5
96	16869	3.8	39.2	8.4
Basal prox. part				
time	cpm	%	%	%
24	220	48.0	15.1	19.0
48	113	30.9	3.8	32.2
72	130	26.9	13.0	28.3
96	105	31.2	14.8	24.0

TABLE 3

A comparison of the R_{NAA}-values, which represent the movement of the unknown compounds relative to NAA, present on the same chromatogram. The *Rf*-values of the different compounds found in our experiments is also given in the table together with the postulated chemical identity of the compounds.

Compound	*Rf*-value	R_{NAA}-value			postulated chem. identity *)
		Klämbt	Zenk	Veen	
A	0.07	—	—	0.11	—
B	0.15	0.23	0.25	0.23	8-OH-1-NAAglu.
C	0.22	0.18	0.28	0.34	NAAsp
C′	0.30	—	0.52	0.47	—
D	0.36	0.65	0.57	0.56	8-OH-NAA
D′	0.40	—	—	0.62	—
D″	0.55	0.75	—	0.86	—
E	0.64	1	1	1	NAA
F	0.87	1.30	1.22	1.36	NAA-glu.
F′	0.93	—	1.22	1.45	—

*) For abbreviations see text.

of compound E is left. The Rf-values of compound E and pure NAA are the same. This strongly suggests that both substances are identical. Another remarkable feature is the rapid increase in the amount of extractable compound C, and to a lesser degree of compound B. Only relatively small amounts are formed of the compounds A, D and F. The decrease in compound E (i.e. NAA) in the tissue and the simultaneous increase in a number of unidentified compounds suggests that NAA is converted to other substances in the tissue.

We next studied the conversion of NAA at different parts of the explants. GORTER (1964) found that the abscission of the petioles of explants was influenced by the length of the proximal parts (above or below the node). This might be explained by assuming a difference between auxin transport and/or metabolism in explants with long or short proximal parts. In view of the results obtained by Gorter we studied the translocation and the metabolism of NAA in explants of which the proximal basal part was 25 mm instead of the usual 5 mm. Donor blocks (2×10^{-4} M) were applied to the apical proximal part. After 24 hours the apical proximal parts (5 mm) and the most basal proximal parts of 10 mm were collected and extracted as described before. After 24 hours the apical proximal parts contained a gross radioactivity of 12000 cpm per mg dry weight, whereas the basal proximal parts only contained 220 cpm per mg dry weight. Chromatographic analysis of the two extracts showed a substantial difference between the ratios of the various compounds in both extracts. Table 2 shows that in the basal part NAA is present as such for nearly 50 per cent (10 per cent in apical part), while compound F is present in a relatively greater amount than compound C, this situation being the reverse of that in the apical proximal part. This difference in metabolic turnover continues for a period of 96 hours (see Table 2). It may be ascribed to the different absolute amounts of radioactivity in the two parts, or it may be an ageing effect (unpublished results); the basal part of the stem is older than the apical part of the stem of the same explant.

Only one of the compounds present in the acetonitrile-soluble fraction has so far been identified. Compound C was hydrolyzed with HCL and chromatographed again. The radioactivity was then recovered at a much higher Rf-value than before treatment and was equal to that of NAA itself, while we could demonstrate a ninhydrin positive spot on the same chromatogram, close to the Rf-value of aspartic acid. Hence compound C is very probably identical with naphthylacetyl aspartic acid. By comparing the data of ZENK (1962) and KLÄMBT (1961) with our own data it is possible to postulate the chemical nature of the other compounds. We therefore calculated the R_{NAA}-values, i.e. the movement of a compound relative to NAA, present on the same chromatogram. These are shown in Table 3. Since the compounds A, B, C, D, E and F together represent more than 90 per cent of the total extracted radioactivity, we will only consider these 6 compounds.

Compound A has not previously been identified.

Compound B might be identical with the glucose ester of 8-hydroxy naphthylacetic acid (8 OH-1-NAAglu). This compound was isolated and identified by KLÄMBT (1961b). The phenolic OH-group is bound as a β-glucoside, so that the molecule has a free acid group like NAA itself.

Compound C is identified as naphthylacetyl aspartic acid (NAAsp). KLÄMBT (1961a) and ZENK (1962) proved the presence of this compound in various plant tissues.

Compound D might be identical with 8-OH-naphthylacetic acid (8-OH-NAA). Like Klämbt, ZENK (1962) assumes that a part of the radioactive NAA is converted into hydroxylated NAA, which may be partly bound to glucose.

Compound E is naphthylacetic acid (NAA).

Compound F, finally, might be identical with naphthyl acetyl β-d-glucose (NAA-glu). This glucose ester was identified by KLÄMBT (1961a) as well as by ZENK (1962).

Some of these compounds are detoxication products, others may be related to growth phenomena. In the discussion attention will be paid to the biological consequences of the observed metabolic turnover of NAA in Coleus explants.

DISCUSSION

No attempt will be made to discuss all the literature relating to auxin transport in plant tissue. The reader is referred to LEOPOLD (1963). It has already been demonstrated (GORTER and VEEN, 1966) that all radioactivity in receiver blocks is from naphthylacetic acid. Therefore it seems logical to assume that the radioactivity in the transport system is not subject to metabolic conversions. One should distinguish between auxin in the transport system and auxin fixed in the cytoplasm and thus withdrawn from the amount of auxin transported. The immobile auxin, so-called auxin pool, consists among other materials, of complexes of auxin with sugars and aspartic acid. It has not yet been established whether or not free NAA still present in tissue in which these complexes are formed is transportable.

Auxin transport in *Coleus* sections is saturated at a donor concentration of 2 to 5 mg per litre (SCOTT and JACOBS, 1963). Their experiments lasted only $2\frac{1}{2}$ hours. After such a short period at least 80 per cent of the acetonitrile-soluble ^{14}C fraction is still present as NAA (see Fig. 13). It therefore seems unlikely that this saturation phenomenon can be explained by the removal of auxin from the transport system by immobilization, as was suggested by GOLDSMITH and THIMANN (1962). Our own theory is that all sites in the "secretion unit" are occupied at a concentration of over 2 to 5 mg per litre. Arguments supporting the concept of such a 'secretion unit' are adduced by HERTEL and LEOPOLD (1963). If this unit is occupied, the auxin concentration in the tissue next to the receiver block will increase and pass a hypothetical threshold value required for enzyme induction. Experiments to prove the presence of a threshold value are now in progress.

The adaptive enzyme system which is formed will conjugate aspartic acid to an auxin molecule by establishing a peptide link between the carboxyl group of the auxin and the amino group of the aspartic acid molecule. The formation of this compound shows a lag period of more than 2 hours (see Fig. 13). The adaptive formation of NAAsp (ZENK, 1962; SÜDI, 1964) means a "de novo" synthesis of an unnatural enzym system. The inducer (NAA) acts catalytically in the formation of the enzyme and not stoichiometrically (DIXON and WEBB, 1960). We may therefore expect a certain donor concentration of the auxin above which induction occurs. Auxin is immobilized in the plant tissue by this enzyme system. This immobilization may explain why after 10 hours no more auxin is secreted out of the tissue into the receiver blocks. The concentration of free auxin in the transport system in the tissue next to the receiver block, decreases to such a low value that no further transport of auxin can be observed.

GOLDSMITH and THIMANN (1962) found that the concentration of radioactivity in a section of an *Avena* coleoptile declines exponentially with the distance from the source. In agreement with these observations we noticed a retention of radioactivity in the first 5 mm below the source up to 70 per cent of the total acetonitrile-soluble ^{14}C in the explant present after 24 hours. The microautoradiographic photographs show that this radioactivity is fixed in the cytoplasm next to the wall. Efforts to localize auxin shortly after application, in an attempt to pinpoint the transport pathway, have so far been unsuccessful so that our results give no definite answer about the route by which auxin is transported in the tissue.

The metabolism of indoleacetic acid was first studied by ANDREAE and co-workers (1955, 1956, 1957 and 1961) in pea root tips. They isolated indoleacetyl aspartic acid and indoleacetamide. The latter product appeared to be an artefact according to KLÄMBT (1961a) and ZENK (1961 and 1962). Besides indoleacetyl aspartic acid, indoleacetyl-β-d-glucose was found in plant extracts (KLÄMBT, 1961a; ZENK, 1961). Klämbt also isolated several hydroxy-auxins viz. 2-hydroxy indoleacetic acid and 8-hydroxy naphthylacetic acid (KLÄMBT, 1959 and 1961b). The β-d-glucosides of these hydroxy-auxins are present to a much larger extent than the free hydroxy-auxins (KLÄMBT, 1964). The author suggests that the hydroxylation may be a prerequisite for the auxin activity in the cell.

As the pattern of the metabolites of indoleacetic acid and naphthylacetic acid is very similar it may be safe to compare these two auxins (KLÄMBT, 1961a).

By comparing R_{NAA}-values we postulated the identity of some of the NAA metabolites formed. We are well aware of the doubts expressed about the real value of this method, but as we used the same solvent system as Zenk and Klämbt a comparison of *Rf*-values is useful.

In agreement with ZENK (1964) the amount of NAAgl (substance F) increases without a lag period, see Fig. 13, in contrast with that of naphthylacetyl aspartic acid, which shows an adaptation period of 2 hours. As we could completely reproduce the findings of ZENK (1964),

we may quote his conclusion here: "the formation of the glucose ester of the auxin seems to be a mechanism for very rapid detoxification of aromatic carbonic acids during a time when the conjugation-system utilising aspartic acid is being adapted in the plant. The glucose ester is not stable in the cell after the aspartic acid-conjugating system has been adapted, but becomes eventually transformed into the auxin-aspartic acid complex. Finally there is a certain amount of free auxin in the cell, which is not exchangeable with auxin in the medium and which is protected from further metabolic alterations." Zenk's last conclusion is supported by the data of Table 2. Even after 4 days still 4 per cent of the total extracted radioactivity is still present as NAA. This percentage is even higher in tissue parts with a very small amount of gross radioactivity. In such a tissue the amount of the auxin-glucose ester is higher than the aspartic acid conjugation, which remains over a period of 4 days, as can be seen in Table 2. We fully agree with Zenk's conclusion that the formation of the glucose ester precedes the auxin-aspartate formation; however, if the gross amount of extracted "auxin" remains very small the glucose ester will be present in larger amounts for a longer period.

Zenk does not assume that the NAA-aspartate conjugation and the glucose-ester formation plays an essential part in the growth induction mechanism. He stated that both conjugates are true detoxication products.

According to KLÄMBT (1964) hydroxy-auxins may be connected with growth phenomena. He stated that the occurrence of hydroxylated auxins in the plant substantiates Muir and Hansch's theory on the mode of action of growth hormones (MUIR and HANSCH, 1953). This concept will not be discussed further.

If compound B is identical with the glucose ester of 8-hydroxy naphthylacetic acid and compound D with the hydroxy-auxin itself, we agree with Klämbt that the glucosides of these auxins are present to a much larger extent than the free hydroxy-auxins themselves (see Figs. 12 and 13).

Although we have no arguments to support the concept of Siegel and Galston on the formation of a complex between auxin and a macromolecule (SIEGEL and GALSTON, 1953), it cannot be rejected. 10 to 15 per cent of the initially applied amount of auxin remains in the tissue after extraction with acetonitrile and with an alcohol-water mixture, and is unidentified. A detailed study of this fraction would seem to be called for.

No attempt will be made at present to relate the results described in this paper to such phenomena as geotropic – and epinastic curvatures, rooting and abscission of petioles, although this will be the goal of further investigations.

ACKNOWLEDGEMENTS

The author is greatly indebted to Dr. D. de Zeeuw for offering him hospitality at the Institute for Atomic Sciences in Agriculture at Wageningen. He also wishes

to thank Miss Dr. C. J. Gorter for many stimulating discussions and for supplying him with material, and Miss M. de Bruin for her technical assistance. The help of Dr. H. Konings in the preparation of the manuscript is also acknowledged.

REFERENCES

ADDICOTT, F. T., R. S. LYNCH, G. A. LIVINGSTON and J. K. HUNTER. 1949. Plant Physiol. **24**: 537.
ANDREAE, W. A. and E. NORMAN GOOD. 1955. Plant Physiol. **30**: 380.
———— and M. W. H. VAN YSSELSTEIN. 1956. Plant Physiol. **31**: 235.
———— and E. NORMAN GOOD. 1957. Plant Physiol. **32**: 566.
————, J. R. ROBINSON and M. W. H. VAN YSSELSTEIN. 1961. Plant Physiol. **36**: 783.
BACH, M. K. 1961. Plant Physiol. **36**: 558.
DIXON, M. and E. C. WEBB. 1960. Enzymes. London.
GALSTON, A. W. and R. KAUR. 1961. Plant Growth Regulation, Iowa State University Press, pag. 355.
———— and W. K. PURVES. 1960. Ann. Rev. Plant Physiol. **11**: 239.
GARDNER, F. E. and W. C. COOPER. 1943. Bot. Gazette **105**: 80.
GIELINK, A. J., G. SAUER and A. RINGOET. 1966. Stain Technology. In press.
GOLDSMITH, M. H. M., and K. V. THIMANN. 1962. Plant Physiol. **37**: 492.
GOOD, N. E., W. A. ANDREAE and M. W. H. VAN YSSELSTEIN. 1956. Plant Physiol. **31**: 231.
GORTER, C. J. 1957. Physiol. Plant. **10**: 858.
————. 1964. Physiol. Plant. **17**: 331.
———— and H. VEEN. 1966. Plant Physiol. In press.
HERTEL, R. and A. C. LEOPOLD. 1963. Planta **59**: 535.
JACOBS, W. P. 1962. Ann. Rev. Plant Physiol. **13**: 403.
————, M. P. KAUSHIK and P. G. ROCHMIS. 1964. Am. Journ. of Bot. **51**: 893.
————. 1965. Plant Physiol. 40 suppl: xxxiii.
KLÄMBT, H. D. 1959. Naturwiss. **46**: 649.
————. 1961a. Planta **57**: 339.
————. 1961b. Planta **57**: 391.
————. 1964. Régulateurs naturels de la Croissance végétale. Paris, pag. 235.
LEOPOLD, A. C. 1963. Brookhaven Symp. in Biology **16**: 218.
LUCKWILL, L. C. 1956. Journ. Hort. Sci. **31**: 89.
MUIR, R. M. and C. HANSCH. 1953. Plant Physiol. **28**: 218.
RUBINSTEIN, B. and A. C. LEOPOLD. 1962. Plant Physiol. **37**: 398.
———— and A. C. LEOPOLD. 1963. Plant Physiol. **38**: 262.
SCOTT, T. K. and W. P. JACOBS. 1963. Science **139**: 589.
SIEGEL, S. M. and A. W. GALSTON. 1953. Proc. Nat. Acad. Sci. **39**: 1111.
STOWE, B. B. 1963. Anal. Biochem. **5**: 107.
SÜDI, J. 1964. Nature **201**: 1009.
THIMANN, K. V. and K. BETH. 1959. Nature **183**: 946.
VENDRIG, J. C. 1960. Wentia **3**: 1.
YARDLEY, H. J. 1964. Nature **204**: 281.
ZENK, M. H. 1961. Nature **191**: 493.
————. 1962. Planta **58**: 75.
————. 1964. Régulateurs naturels de la Croissance végétale. Paris, pag. 241.