# STUDIES ON PHLOEM EXUDATION FROM YUCCA FLACCIDA HAW. X. TRANSLOCATION OF INDOLE-3-ACETIC ACID

H. VEEN and P. M. L. TAMMES

Centrum voor Plantenfysiologisch Onderzoek, Wageningen[1]

SUMMARY

An investigation was made whether indoleacetic acid-l-$^{14}$C can be translocated through the phloem of segments of the apical part of the young inflorescence of *Yucca flaccida* Haw. The radioactivity in phloem exudate from wounds was used as a criterium for translocation. A polar movement (more upwards than downwards) could be observed during the first hours of the experiments; later the concentration of $^{14}$C in the exudate became equal in both directions. The relative radioactivity of the exudate was much lower with indoleacetic acid than with substances that are readily translocated through the phloem, e.g., sucrose or maleic hydrazide. Thin-layer chromatography of the exudate showed the presence of two major radioactive metabolites different in Rf from indoleacetic acid and an almost complete absence of indoleacetic acid itself.

## 1. INTRODUCTION

In recent years a number of papers have been published on the transport of auxins in isolated plant segments. Less attention has been given to problems of auxin transport in intact plants (MORRIS *et al.* 1969, MORRIS 1970). Using autoradiographic techniques it has been shown that applied indoleacetic acid (IAA)–$^{14}$C) shows a similar distribution to assimilates moving in the phloem (FANG & BUTTS 1957). Further evidence that auxin is transported in the phloem was presented by the experiments of ESCHRICH (1968) using aphids to analyse the phloem sap. He showed that only IAA was present in the phloem after applying radioactively labelled IAA to the leaf, although in plant tissue extracts two other labelled compounds were isolated. This is strikingly similar to auxin transport in plant segments, in which also the auxin molecule is the sole mobile component. We decided therefore to study transport of IAA in phloem using the exudation technique described in detail by VAN DIE & TAMMES (1966) and TAMMES, VONK & VAN DIE (1967).

## 2. MATERIAL AND METHODS

Stalks about 50 cm long of young inflorescences of *Yucca flaccida* Haw. growing outdoors were collected and stored in the dark at 2 °C. Under such conditions they do not bleed. An hour before the experiment started the stalks were brought to room temperature with the basal end in a small beaker with water to refill the

[1] Address: Centre for Plant Physiological Research, Bornsesteeg 47, P.O.Box 52, Wageningen, The Netherlands.

xylem vessels with water to compensate turgor deficit. Subsequently a 15 cm piece was cut out of the stalk and was placed with one end in a small beaker containing 2 ml of a solution of labelled IAA.

Indole-3-acetic acid -1-$^{14}$C (IAA-$^{14}$C) with a specific activity of 25.6 mCi/mmole was purchased from the Radiochemical Centre in England. Its purity was checked by thin-layer chromatography, using silica gel as a layer and isopropanol-ammonia-water as solvent system. No more than 20% of the total radioactivity was present on the chromatogram at any Rf zone other than that of IAA. The IAA was dissolved in de-ionized water and used at various concentrations ranging from 2 μM to 200 μM. From the opposite end of the stalk segments the exudate was collected with small calibrated pipettes (Drummond Microcaps) In the first 150 min of the experiments the sap was collected every 15 min, thereafter every 30 min. In some experiments the collected phloem sap was frozen in solid carbon dioxide and stored in a freezer for identification afterwards. In other experiments the amount of sap exuded was measured and subsequently blown out into a small filter-paper disc which was then transferred into a counting vial to which was added 10 ml of a scintillation fluid based on dioxane and naphthalene (VEEN 1967). The vials were counted in a liquid scintillation spectrometer.

Labelled compounds in sap and in tissue parts cut from the segments after the experimental period were identified by thin-layer chromatography. The exuded sap was spotted on to the silica layer. The 15 cm stalk segment was cut into 5 pieces each 3 cm long, which were frozen in solid $CO_2$. The pieces were then ground in a mortar and extracted for 48 hours with 25 ml acetonitrile at 4°C in the dark. The homogenate was centrifuged to remove debris. The acetonitrile extract was reduced in volume by evaporating in a vacuum oven and spotted onto a silica layer. The chromatograms were developed with isopropanol, 25% ammonia solution and water (8:1:1). The chromatograms were covered with a Melinex polyester film to avoid chemical reactions with the film plate. A Kodak medical X-ray no-screen film was then placed against the chromatogram. The film was exposed for about 4 weeks. In some experiments the chromatogram plates were sprayed with an intensifier (Omnispray, NEN) before placing the film against the layer to increase the sensitivity of the technique. If so, the films were exposed for one week to solid $CO_2$. The radioactivity in the tissue extracts was measured by taking samples and counting radiations in the scintillation counter. Quenching by chlorophyll was corrected with an automatic external standard (AES) in association with an AES ratio/efficiency curve. Each experiment was run 2 or 3 times unless otherwise specified.

## 3. RESULTS

### 3.1. Time course of translocation of $^{14}$C from IAA-$^{14}$C

The amounts of sap exuded vary greatly between segments and for any segment according to its orientation, e.g., upright and inverted. In a typical experiment 6 segments were used; three segments were placed with their basal end down in a

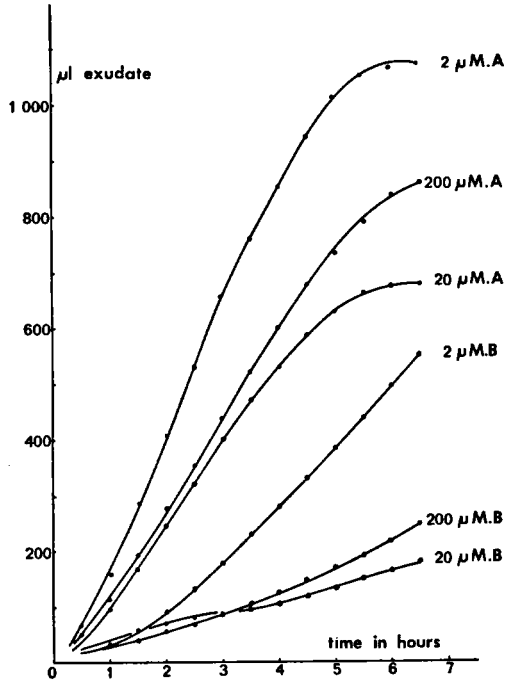


Fig. 1. Time course of the exudate volume at three different initial auxin concentrations. Segments were oriented with their basal end down (A, acropetal movement) or with their apical end down (B, basipetal movement).

beaker (acropetal movement), three other segments were placed upside down (basipetal movement). The beakers contained 2 ml of a radioactive solution of IAA in three different concentrations: 200, 20, and 2 µM. *Fig. 1* shows that in the natural position (basal end down) a slow but constant bleeding occurs during the first five hours. Thereafter exudation decreases and after 7 hours stops. According to VAN DIE & TAMMES (1966) flow was renewed after cutting a thin slice from the wound surface. In the experiments now described no slices were cut. In the inverted position less sap was exuded but continued to flow after 5 hours. The data of *fig. 1* further suggest a relationship between the concentration of IAA in the external solution and the amounts of sap exuded but in two identical experiments this could not be verified because of the great variability between segments. The 2 ml of radioactive solution is sucked into the segments after about 150 min. Before the cut end dried up de-ionized water was added to the beaker.

The radioactivity of the exudate was measured. After placing a segment in the radioactive solution in an upright position, radioactivity was found in the exudate within 30 min with the highest concentration of auxin. In the inverted position this time lapse is about 75 min. At a low concentration of 2 µM radioactivity can be found in the exudates only after 60 min in the upright position and after 150 min in the inverted. As the amount of fluid exuded varied so much, the $^{14}C$ data are presented as counts per minute per 100 µl sap. The data in *fig. 2*

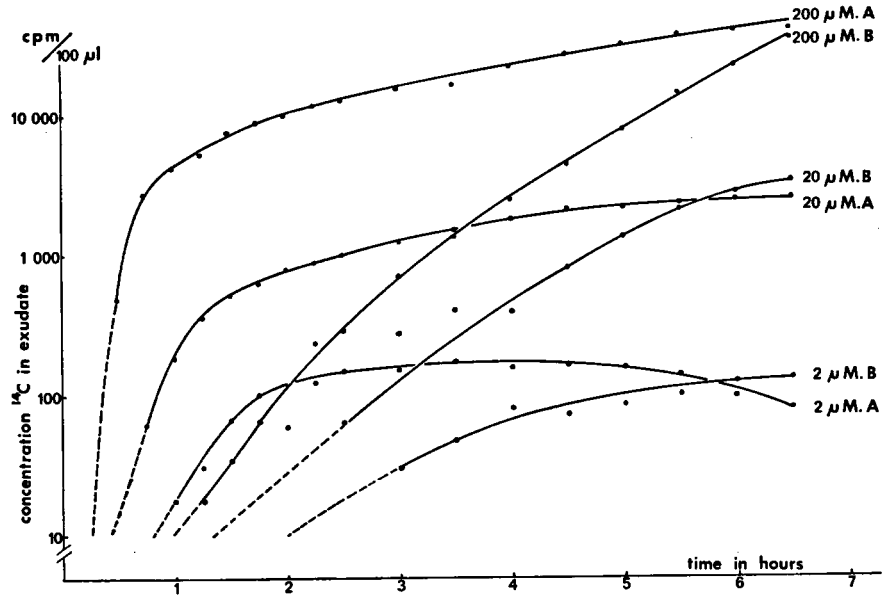


Fig. 2. Time course of translocation of $^{14}C$ from indole-3-acetic acid at three different initial concentrations. A, acropetal movement (segments upright). B, basipetal movement (segments inverted). Note that the y axis is logarithmic.

show that, if the auxin concentration in the external solution is ten times as high, the concentration in the bleeding sap is also about tenfold (note that in *fig. 2* the y axis is plotted logarithmically). The relationship between time and $^{14}C$ concentration in the exudate is characteristic for the upright and for the inverted positions. For upright segments with acropetal translocation $^{14}C$ concentration increased rapidly. Concentration reached a maximum after 210 min with the lowest concentration of auxin. With higher concentrations the maximum is reached after about 360 min. In basipetal direction the increase in $^{14}C$ concentration was much lower and does not reach the maximum within the experimental period. Despite the less vigorous translocation of $^{14}C$ in basipetal direction (inverted segments) during the first period, the concentration of $^{14}C$ in the sap equals the concentration in upright segments at the end of the experimental period. To clarify the relationship between the initial concentration of auxin and the $^{14}C$ concentration in the exudate, *figs. 3* and *4* give the values obtained by dividing the $^{14}C$ concentration in the exudate by the original concentration ($C/C_o$). In *fig. 3*, showing acropetal movement during the first 210 min, the increase in $^{14}C$ concentration in the sap was independent of the initial concentration. Thereafter $C/C_o$ was clearly dependent on the initial concentration. At the lowest concentration less $^{14}C$ is exuded, yet the total amount of exuded $^{14}C$ was not even 1 % of the amount absorbed by the tissue. This suggests an immobilization of the radioactive material inside the plant segment. At

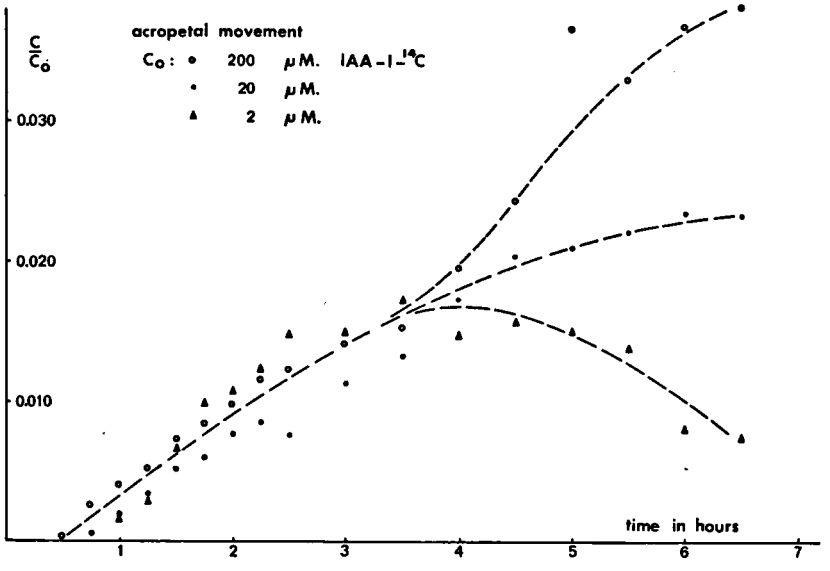


Fig. 3. Relationship between time and $C/C_0$ at three different initial auxin concentrations. C = $^{14}C$ concentration in the exudate; $C_0$ = initial $^{14}C$ concentration in the solution. Acropetal movement.

higher concentrations $^{14}C$ in the exudate continued to increase slowly but there also immobilization eventually stopped exudation of radioactive material. The flattening of the curve for the lowest concentration of auxin in *fig. 3* was not related to a decreased exudation of sap.

With basipetal movement also (*fig. 4*), the increase in translocation of $^{14}C$ was independent of the concentration in the first period of the experiments. Later the $C/C_0$ was considerably less at lower concentrations, indicating that radioactivity became fixed in the tissue. Hence $C/C_0$ can be used as a parameter for the translocation capacity of the system. In some preliminary experiments these ratios were calculated for the translocation of sucrose -$^{14}C$ and of maleic hydrazide –2, 3-$^{14}C$. (MH). Both compounds are mobile in phloem elements (CRAFTS 1964, TAMMES *et al.* 1967). The data are given in *table 1*. They indicate that IAA is much less mobile than sucrose or MH. A breakdown of the IAA-$^{14}C$ or an immobilization in the tissue could explain the phenomenon. To check this hypothesis, extracts of plant tissue were chromatographed.

### 3.2. Identification of $^{14}C$ in phloem exudate and tissue

By chromatography of the exudate some radioactive compounds were detected (*fig. 5a* and *b*). Usually only phloem sap exuded between the 120th and 240th min was analysed. The exudate of the first hour might still be contaminated with cell fluids from the cut surface and was discarded. The identity of compounds A, B, C and D is unknown. The Rf value of compound D was identical with

Table 1. $C/C_o$ for transport of three different compounds. Acropetal translocation (A) and basipetal translocation (B) have been studied. The ratios were calculated after 210 and 330 min. MH = Maleic hydrazide.

| Treatment | Time | | | |
|---|---|---|---|---|
| | 210 min | | 330 min | |
| | A | B | A | B |
| 200 μM IAA* | 0.016 | 0.001 | 0.033 | 0.014 |
| 20 μM IAA* | 0.013 | 0.004 | 0.022 | 0.022 |
| 2 μM IAA* | 0.017 | 0.005 | 0.014 | 0.010 |
| 5 μM sucrose | 0.230 | 0.055 | 0.370 | 0.065 |
| 707 μM MH | 0.260 | 0.032 | 0.500 | 0.076 |

* data from figures 3 and 4

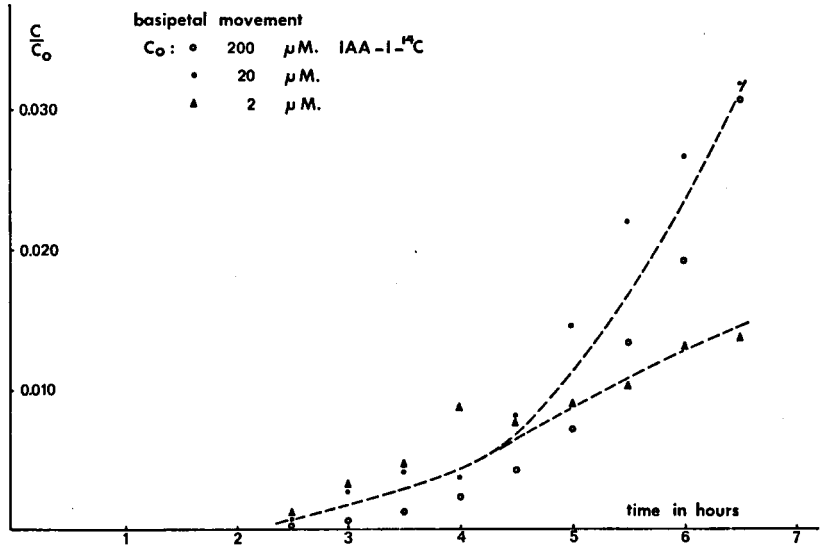


Fig. 4. Legend the same as for figure 3. Basipetal movement.

that of IAA. If compound D is IAA, only a very small proportion of the radioactivity has remained in the original form. These results contrast with those of ESCHRICH (1968).

The distribution of the $^{14}C$ in the whole segment was studied by extraction. The 15 cm long stalk segment was cut into five parts 3 cm long. These parts were extracted as described earlier. When the total radioactivity of the extract was plotted against the position of the various segment parts, a linear logarithmic decrease in radioactivity within the stalk segment with distance from site of uptake could be observed (*fig. 6*). This is a well-known phenomenon in transport

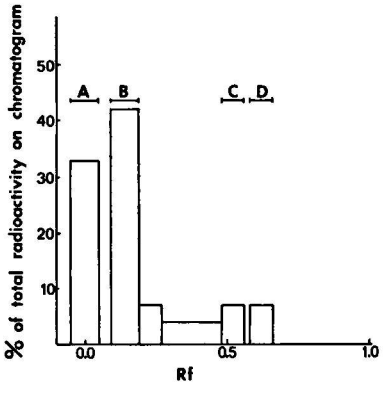


Fig. 5a. Distribution of radioactivity in a thin-layer chromatogram of exudate. The radioactivity is calculated as a percentage of total radioactivity present on the chromatogram. Total corrected counts per min were 1065.

studies (GOLDSMITH & THIMANN 1962, MORRIS 1970). It fits the model of HORWITZ (1958), in which irreversible removal of activity from the transport system establishes the logarithmic distribution. In some other experiments of ours a deviation from such a linear relationship was found in the most distant parts of the segment (compare GOLDSMITH & THIMANN 1962, *fig. 7*). By adding the total activity extracted from the 5 parts and the total radioactivity in the

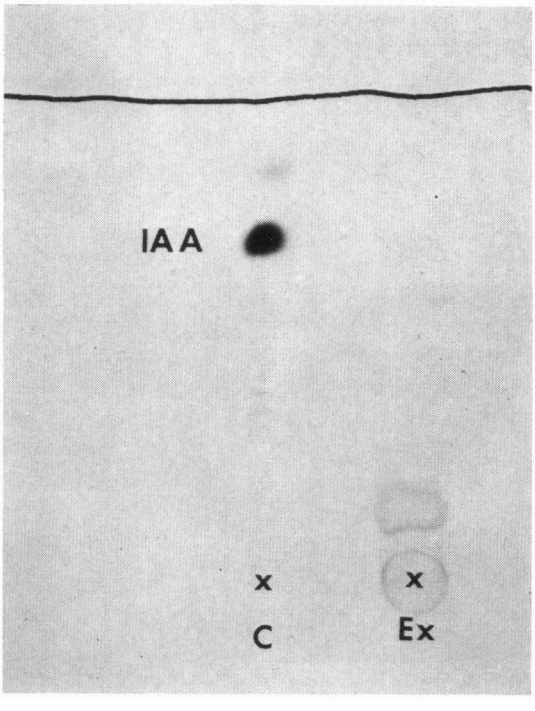


Fig. 5b. Photograph of a Kodak No-screen X-ray medical film that had covered a thin-layer chromatogram. In exudate (Ex) collected between 150 and 240 min after the start of the experiment two major metabolites at low Rf were recovered. A control run of IAA-1-$^{14}$C is also shown (C). The initial concentration of IAA in this experiment was 200 $\mu$M.

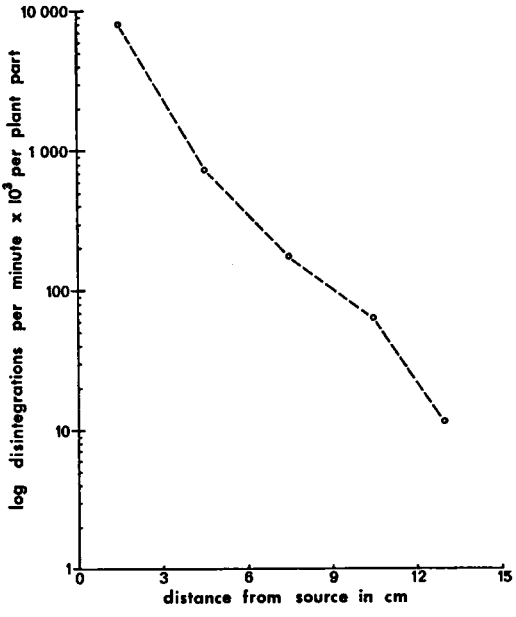


Fig. 6. Relationship between the amount of radioactivity present in different parts of the segment and the position of the successive parts in the segment judged by their distance from the site of uptake. Radioactivity is plotted at the midpoint of the part of the segment. Transport period: 270 min. acropetal translocation. Initial concentration: 200 μM.

exudate, a percentage recovery was calculated. In such a calculation it was assumed that all applied radioactivity was absorbed by the tissue. The recovery in our experiments was low, not more than 40%, which indicates a) the formation of acetonitrile-insoluble compounds, b) the release of radioactive carbon dioxide by enzymic or non-enzymic processes (IVERSEN & AASHEIM 1970, MANN & JAWORSKI 1970). Our recovery percentages correspond with those of VEEN & JACOBS (1969) in experiments on auxin transport in *Coleus* segments. The various tissue extracts were chromatographed. The ratio between the amounts of different compounds in the chromatograms depends greatly on the position of the part in the segment (*table 2*). The data in *table 2* suggest that less IAA is present in the parts of the segment further from the site of uptake. In this way the chromatograms of exudate resemble the chromatograms of the segment part where sap exudes. The nature of the radioactive compounds at lower Rf values is unknown, indoleaspartic acid in this solvent system also shows a low Rf (VEEN & JACOBS 1969). The film plates covering the chromatograms show, however, that there is more than one compound there.

## 4. DISCUSSION

The experiments show that IAA-$^{14}$C can be translocated through the phloem. This translocation is initially polar (more acropetal than basipetal), later translocation is equal in both directions, as judged by data on specific activity (counts $min^{-1}$/volume exudate). Anyway it is a non-specific polar movement, as sucrose

Table 2. The relationship between auxin content expressed in disintegrations per min (dpm) and metabolism in acetonitrile extracts after translocation for 330 min. Basipetal translocation. Concentration 100 μM. Radioactivity in different areas of the chromatograms expressed as a percentage of total activity on the chromatograms.

| Segment part | dpm extr. x $10_2$ | Rf value | | | |
|---|---|---|---|---|---|
| | | 0.0–0.32 | 0.32–0.52 | 0.52–0.75 | 0.75–1.00 |
| 1 | 2.7 | 32.4 | 51.0 | 9.0 | 7.6 |
| 2 | 1.8 | 34.8 | 35.9 | 17.3 | 12.5 |
| 3 | 4.2 | 27.2 | 33.9 | 29.7 | 8.8 |
| 4 | 129.2 | 11.0 | 4.9 | 75.3 | 8.7 |
| 5 | 2412.6 | 11.5 | 3.4 | 76.0 | 9.0 |
| IAA-1-$^{14}$C(control) | | 6.2 | 3.1 | 83.8 | 6.9 |

and maleic hydrazide show a similar pattern of translocation. It might be that anatomic differences between the lower and upper cut ends cause the initial difference in movement.

The data on the effects of the initial auxin concentration on the $^{14}$C concentration in the exudate show clearly that at least during the first hours transport is almost proportional to concentration. Earlier VEEN (1967) observed that in *Coleus* explants the amount of radioactivity reaching the receiver blocks did not increase linearly with auxin concentration in donor blocks. The latter data have been used by GOLDSMITH (1969) as evidence that a specific transport site is involved, analogous to the behaviour of an enzymatic reaction. The translocation of $^{14}$C in the present system may be completely different from the system earlier studied in *Coleus* explants. Chromatographic studies showed that the $^{14}$C in the exudate is almost all in compounds other than IAA. This is in contrast with the data of ESCHRICH (1968) obtained by a different technique with aphid stylets and with other plant material: entire young plants of *Vicia faba* with IAA applied to the first primary leaf.

LEPP & PEEL (1971) also investigated the phloem mobility of IAA. They applied IAA-$^{14}$C to willow bark by abrasion. Honeydew from aphids showed considerable radioactivity. It was, however, not clear whether the radioactivity of honeydew was in IAA only or in metabolites of IAA or both. The metabolites detected in the exudate might be formed in the phloem or might be secreted from surrounding tissue into the phloem cells. Incubation experiments in which IAA-$^{14}$C was incubated with phloem sap were unsuccessful: there was no indication whatever that any metabolite was formed. But the phloem sap used in this experiment was collected two years earlier and had been stored in a freezer at –20 °C.

Segment parts through which $^{14}$C was translocated also showed rapid metabolism of IAA. This turn-over seemed more intense further from the site of uptake. These data are difficult to interpret as we cannot trace the pathway of the $^{14}$C through the segment. The radioactive solution is probably sucked by the segments into the xylem vessels by capillary forces. Then there is a lateral move-

ment into the phloem cells, followed by a pressing out of the content of the sieve tube onto the cut surface. The wound at the opposite surface was closed by itself by storing the segment one night over in the dark at 2 °C. Arguments that this is a real phloem secretion have been presented earlier by VAN DIE (1968). He argues on anatomic and cytological grounds that the sap originates from sieve tubes. Also the analytical data – high sucrose content, absence of free hexoses, high potassium content, low calcium content and an alkaline pH – demonstrate that the exudate originates from sieve tubes. The origin of the exudate in phloem was confirmed by experiments in which radioactive carbon applied as $^{14}CO_2$ to a mature leaf appears as labelled sucrose in the exudate (VAN DIE & TAMMES 1964). Earlier VEEN (1967) argued that a closer analysis of auxin transport in the whole plant as opposed to that in sections was needed. How far the observed phenomena on translocation and metabolism of auxin reflect a natural pathway of endogenous auxin and its turn-over remains completely obscure.

ACKNOWLEDGMENT

The authors are indebted to Miss M. de Bruin for technical assistance and to Mr J. C. Rigg (Pudoc Wageningen) for correction of the English text.

REFERENCES

CRAFTS, A. S. (1964): Herbicide behaviour in the plant. In: L. J. AUDUS (ed.). *The Physiology and Biochemistry of Herbicides*: 75–110. London. Academic Press.

DIE, J. VAN (1968): The use of phloem exudates from Yucca flaccida Haw. in the study of translocation of assimilates. *Vorträge aus dem Gesamtgebiet der Botanik* N.F. **2**: 27–30.

— & P. M. L. TAMMES (1964): Studies on phloem exudation from Yucca flaccida Haw. II. The translocation of assimilates. *Acta Bot. Neerl.* **13**: 84–90.

— & — (1966): Studies on phloem exudation from Yucca flaccida Haw. III. Prolonged bleeding from isolated parts of the young inflorescence. *Proc. Kon. Ned. Akad. Wetensch. Amsterdam* C**69**: 648–654.

ESCHRICH, W. (1968): Translokation radioaktiv markierter Indolyl-3-Essigsäure in Siebröhren von Vicia faba. *Planta (Berl.)* **78**: 144–158.

FANG, S. C. & J. S. BUTTS (1957): Studies of carboxyl-$^{14}$C-labeled 3-indoleacetic acid in plants. *Plant Physiol.* **32**: 253–259.

GOLDSMITH, M. H. M. (1969): Transport of plant growth regulators. In: M. B. WILKINS (ed.): *The Physiology of Plant Growth and Development*: 127–162. London. McGraw-Hill.

— & K. V. THIMANN (1962): Some characteristics of movement of indoleacetic acid in coleoptiles of Avena. I. Uptake, destruction, immobilization, and distribution of IAA during basipetal translocation. *Plant Physiol.* **37**: 492–505.

HORWITZ, L. (1958): Some simplified mathematical treatments of translocation in plants. *Plant Physiol.* **33**: 81–93.

IVERSEN, T.-H. & T. AASHEIM (1970): Decarboxylation and transport of auxin in segments of sunflower and cabbage roots. *Planta (Berl.)* **93**: 354–362.

LEPP, N. W. & A. J. PEEL (1971): Patterns of translocation and metabolism of $^{14}$C-labelled IAA in the phloem of willow. *Planta (Berl.)* **96**: 62–73.

MANN, J. D. & E. G. JAWORSKI (1970): Minimizing loss of indoleacetic acid during purification of plant extracts. *Planta (Berl.)* **92**: 285–291.

MORRIS, D. A. (1970): Light and the transport and metabolism of indoleacetic acid in normal and albino dwarf pea seedlings. *Planta (Berl.)* **91**: 1–7.

—, R.E. BRIANT & P. G. THOMSON (1969): The transport and metabolism of $^{14}$C-labelled indoleacetic acid in intact pea seedlings. *Planta (Berl.)* **89**: 178–197.

TAMMES, P. M. L., C. R. VONK & J. VAN DIE (1967): Studies on phloem exudation from Yucca flaccida Haw. VI. The formation of exudate-sucrose from supplied hexoses in excised inflorescence parts. *Acta Bot. Neerl.* **16**: 244–246.

VEEN, H. (1967): On the relation between auxin transport and auxin metabolism in explants of Coleus. *Planta (Berl.)* **73**: 281–295.

— & W. P. JACOBS (1969): Transport and metabolism of indole-3-acetic acid in Coleus petiole segments of increasing age. *Plant Physiol.* **44**: 1157–1162.