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QUANTITATIVE STUDIES ON TRANSLOCATION IN SAGITTARIA GRAMINEA MICHX. LEAVES, A NEW METHOD OF MEASUREMENT

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SUMMARY

A scanning method is described for measuring the uptake of a labeled compound and the subsequent distribution of this compound or its conversion products over a *Sagittaria* leaf.

The measurement can be repeated several times after the leaf has been extracted with alcohol/aceton, solutions of proteolytic enzymes, dilute acids etc., giving exact data for the distribution of various groups of substances over the length of the leaf. The usefulness of this method is illustrated by some experiments on the uptake and translocation of amino acids in light and in darkness. The results obtained so far indicate a polar, non-specific translocation process, in which the metabolic role of the translocated amino acid seems of minor importance.

1. INTRODUCTION

The uptake and translocation of inorganic ions and amino acids in submerged leaves has been studied extensively by Arisz and collaborators. Using *Vallisneria* leaves they measured translocation either by direct assay of a limited number of leaf sections (ARISZ 1953) or by autoradiography (ARISZ 1960). Although the first method may give accurate quantitative results on the distribution of a substance, especially when large numbers of sections are analysed, it has the disadvantage of being a laborious and time-consuming procedure. Measuring translocation by autoradiography is more generally used, but this method only allows a rough estimation of the distribution of the label over the leaf. Neither does it give quantitative information on the degree of metabolic conversion of the labeled substance in the leaf during the experiment.

BELIKOV (1955), in translocation studies on soybeans, used direct Geiger-Müller counting of leaf discs following various extraction procedures. This method has been adopted and refined in the present study, in which a radiochromatogram scanner is used to measure the distribution of radioactivity over the whole of *Sagittaria* leaves, which because of their thinness and shape are very suited for scanning.

In the present paper a description is given of some experiments on amino acid uptake and translocation, in which this scanning technique has been applied.

2. MATERIAL AND METHODS

Leaves of *Sagittaria graminea* Michx. were cut to a length of about 20 cm and placed in distilled water for about 18 hrs. They were subsequently transferred to a flat perspex box the bottom of which was covered with a layer of 2% agar. A small agar block of 7 × 3 × 1 mm, containing the radioactive substance to be absorbed, was put on either the tip or the basal end of each leaf. The remaining part of each leaf was covered with moistened 'Kleenex' tissue in order to keep the leaves wet. After a number of hours the agar blocks were removed and the leaves were rinsed in tap water before being freeze dried at –20°C or even lower temperatures in a 'Martin Christ' freeze dryer for about 24 hrs. The dried leaves were mounted between 'melinex' (I.C.I.) 8 microns in thickness in a slit cut in a paper strip. This strip was scanned in a Packard radiochromatogram scanner with a speed of 1 cm/min, using helium/isobutane as a counting gas.

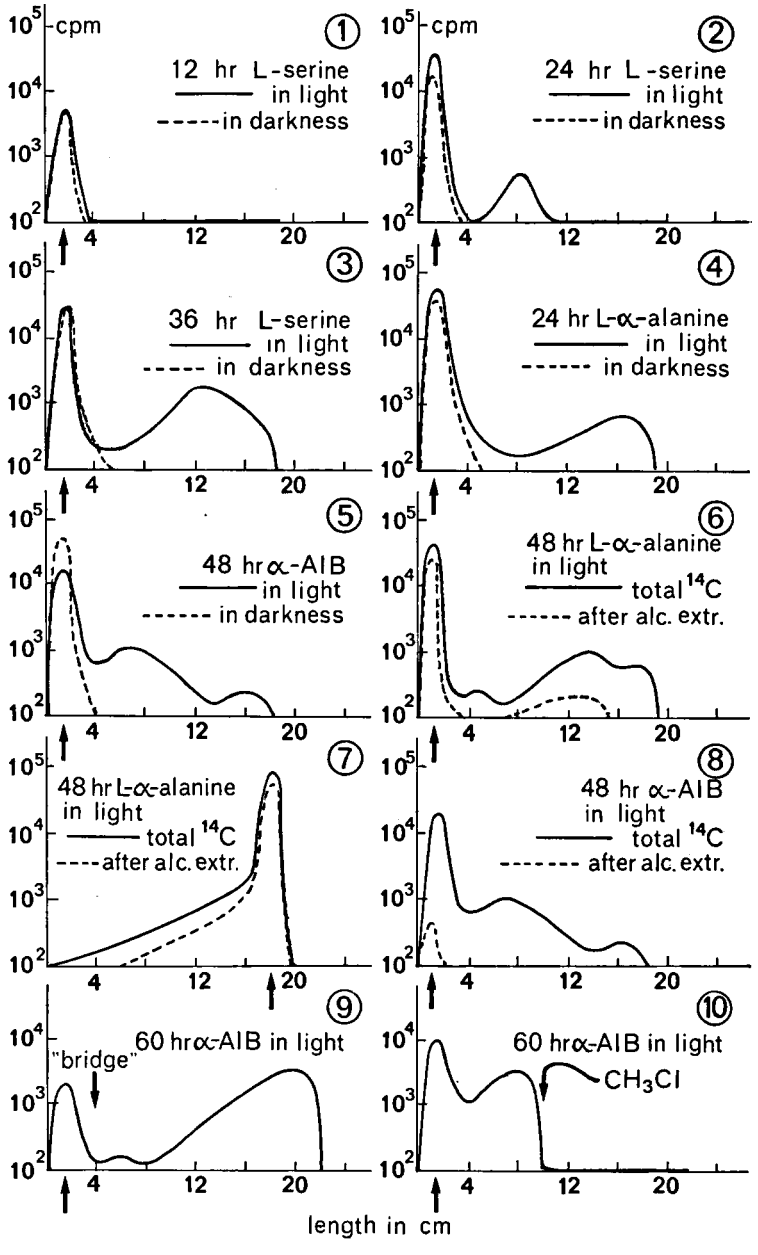
After being scanned some leaves were removed from the paper strip and placed into a cylinder filled with 80% ethanol and kept near boiling temperature till the green pigments were completely extracted. They were put into a cylinder with acetone for some time and then carefully dried between layers of filter paper under slight pressure. A repeated scanning of the leaf gave the distribution of the radioactivity of the ethanol insoluble substances over the length of the leaf. Similarly scanning of the leaf can be repeated after removal of other substances, e.g. proteins removed with a protease solution, and certain polysaccharides removed either with dilute acids or amylase.

All radioactive amino acids used were obtained from the Radiochemical Centre, Amersham, England.

3. RESULTS AND DISCUSSION

The time courses of the uptake and translocation of some amino acids in light and in darkness are shown in *figs. 1–5*. The results clearly demonstrate that both the uptake and the translocation increase with time. The translocation process, however, appears to be enhanced more strongly than the uptake is. *Figs. 1–5* also show that in darkness hardly any translocation takes place, but that light promotes this process very much. On the other hand, no distinct light effect on the uptake could be detected. The results are partly in accordance with the view of ARISZ (1960) based on his experiments with *Vallisneria*: Uptake and translocation are different processes and light has a stimulating effect on translocation only. The uptake of amino acids in *Sagittaria*, however, was not stimulated by light, as contrasted with the uptake of ions in *Vallisneria* (ARISZ & SOL 1956; VAN LOOKEREN CAMPAGNE 1957).

The present experiments also revealed an unexpected accumulation of amino acids in the basal regions of the leaf when the amino acid was absorbed by the leaf tip (*figs. 1–6*). This phenomenon could not be observed if the labeled substance had been applied to the basal part of the leaf (*fig. 7*). This phenomenon cannot be attributed to local anatomical differences, as in short leaf segments



Figs. 1–10. Time-courses of the uptake and translocation of some ^{14}C-amino acids under various conditions. Only in the experiment of *fig. 7* the label was put on the cut basal end of the leaf. Sites of application are indicated by vertical arrows.
α-AIB = α-aminoisobutyric acid.

(approximately 10 cm) as well as in longer ones (approximately 30 cm) the accumulation appeared in the most basal parts of the leaf segments. It rather looks like a congestion of substance, which cannot pass a barrier, e.g. the end of the leaf (segment), or, as shown in *fig. 10*, a small part halfway up the leaf, killed by a droplet of chloroform. The last experiment also demonstrates that a continuity of living cells is a prerequisite for translocation. The polarity in distribution of the amino acid over the leaf, as shown in *figs. 6* and *7*, is not the result of damage at one end of the leaf, as leaves with severed tips behave similar to controls with intact tips.

In accordance with the findings of ARISZ & SCHREUDER (1956) on asparagine translocation in *Vallisneria*, in *Sagittaria*, too, the number of living cells participating in the translocation process does not simply act as a limiting factor for its rate: translocation through a narrow bridge of leaf tissue proceeds virtually unhampered as compared to intact leaves (*fig. 9*).

Figs. 8 and *9* show the uptake and translocation of α-aminoisobutyric acid (AIB). AIB is not converted by *Sagittaria* leaves into proteins or other cell constituents and is therefore an interesting substance in the study of amino acid translocation. It appears to be normally translocated in the leaf, just like the protein amino acids L-α-alanine and L-serine (MOTHES & ENGELBRECHT 1961). This observation allows the important conclusion that the uptake and translocation systems for amino acids in *Sagittaria* with its typical polar distribution pattern and its enhanced light-dependent accumulation cannot be regarded as specific processes.

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