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# PATHWAYS OF TRANSLOCATION AND METABOLIC CONVERSIONS OF ROOT-ABSORBED $^{14}C$ (U) L-GLUTAMIC ACID IN TOMATO PLANTS

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ABSTRACT

$^{14}C$ (U) L-glutamic acid was supplied to the roots of young tomato plants. After a four hours' uptake period all the amino acids present in the root proteins were weakly labelled, but of the free amino acids of the roots only $\gamma$-aminobutyric acid, glutamine, glutamic acid and aspartic acid proved to be active. In addition, the label was incorporated into lipoids and a number of carboxylic acids.

The extent of labelling of the various leaves appeared to be correlated with the synthetic activities of these leaves, and inversely related with leaf age. The distribution was not influenced by the presence or absence of roots.

It is concluded that the xylem is the initial way of translocation of amino acids, which are synthesized in the roots. During their movement through the xylem vessels to the areas of transpiration, the amino acids are partly absorbed by surrounding tissues and translocated to centres of protein synthesis.

## INTRODUCTION

Previous studies have shown that tomato roots synthesize a number of amino acids and amides, especially if ammonium is available as a nitrogen source. These compounds are partly translocated to the aerial parts of the plant [VAN DIE, 1958, 1959, 1960, 1961]. In general it is believed that these amino compounds move in the xylem stream. Evidence for this view was gained from analyses of bleeding sap as well as from analyses of xylem sap obtained from short pieces of stem [e.g. BOLLARD, 1957, 1960].

The question arises how far the amino acid movement in an upward direction is mainly or exclusively through the xylem vessels.

In an interesting paper JOY (1962) suggested the existence of different pathways of translocation for protein amino acids and non-protein amino acids. According to him, this differentiation presumably implies the existence of a mechanism by which the different types of amino acids are recognized and segregated in the roots.

The present paper reports studies on the fate of $^{14}C$-L-glutamic acid after its uptake by the roots. In short-term experiments the metabolic conversions of this amino acid and the distribution of the label in the tomato plant have been examined. Evidence is presented for the view that the xylem is the initial way of translocation of the glutamic acid, but that superimposed on, or concurrently with this movement through the xylem vessels, other translocation mechanisms are also active.

## METHODS

### *Plants*

The tomato variety used in former investigations ("Ailsa Craig") was also used for the present experiments. The plants were grown in the greenhouse in waterculture using the nutrient solution of HOAGLAND and BROYER (1936).

### *Radioactive glutamic acid*

The randomly Carbon-14 labelled monoammonium salt of L-glutamic acid was employed. It was obtained from the Radiochemical Centre, Amersham, Great Britain. Its purity was chequed by paper chromatography.

### *Extraction*

The plant parts were thoroughly washed, separately ground in an all-glass homogenizer and extracted with 80 per cent ethanol (three times), 96 per cent ethanol (twice) and ethanol-chloroform (2:1; twice). The total extracts were reduced to a few ml by destillation *in vacuo* at room temperature. The substances present in the solution were subsequently partitioned between chloroform and water. The chloroform layer was again extracted with an equal volume of water and both the aqueous and chloroform solutions were reduced to a convenient volume. The insoluble residue was dried at room temperature and either used for determination of radioactivity or used for protein extraction.

### *Protein separation*

The non-soluble material was washed with cold 0.5 N perchloric acid, dissolved in N sodium hydroxide and allowed to digest at room temperature for at least 1 hour to remove RNA. After acidification with 6 N hydrochloric acid, the protein was centrifuged down and re-extracted with N sodium hydroxide. The protein was reprecipitated and boiled for 5 minutes with 0.5 N perchloric acid. The protein was treated with ethanol-ether (1:2) to remove traces of lipids and afterwards dried by two washes of ether-light petroleum (3:1). The dried protein was hydrolyzed in 6 N hydrochloric acid in a sealed tube at 110° C for 16 hours. Residual "humin" was removed by centrifugation and the supernatant was evaporated to dryness to remove the hydrochloric acid. Afterwards the amino acids were separated by paper chromatography.

### *Chromatography*

The water-soluble substances were concentrated by freeze-drying and separated by descending two-directional paper chromatography. Tertiary butanol-formic acid-water (695:10:295) was used as the first solvent and phenol-water-ammonia (155:43:2, W/V/V) as the second (ROCKLAND and UNDERWOOD, 1954).

*Determination of radioactivity*

Aliquots of solutions and suspensions were dried in planchets and counted with an end-window G.M. tube (1.6 mg per $cm^2$), using the "infinitely thin" method. Radioautographs were made from the active chromatograms by placing "Kodirex" X-ray film on the chromatogram and developing after an exposure period of 2–4 weeks. The activities of the various amino acids were measured directly on the chromatograms.

Specific activities of the leaves were expressed in counts per minute (cpm) per gram fresh weight. The fresh weight was believed to be the most convenient basis for these calculations as it appeared directly proportional to leaf area.

## Results

*Uptake and distribution of glutamic acid*

A solution containing 2 mg of $^{14}C$ (U) L-glutamic acid (12.5 $\mu c$), 20 ml of $10^{-3}$ M ammonium bicarbonate and 20 ml of nutrient solution was supplied to plants with about 6–8 leaves. After 4 hours the plants were removed from the solution and their roots thoroughly washed. The leaves, stems and roots were separately analysed. From

Table 1

The distribution of radioactivity in various parts of a young tomato plant, 4 hours after $^{14}C$ (U) L-glutamic acid was supplied to the root system.

| Leaf no. | Fresh wt. gr | % of total leaf area | cpm | % of total leaf activity |
|---|---|---|---|---|
| 1 (oldest) | 0.47 | 10.5 | 2300 | 3.8 |
| 2 | 0.73 | 16.4 | 3650 | 6.0 |
| 3 | 1.71 | 38.4 | 21460 | 35.5 |
| 4 | 1.05 | 23.6 | 18160 | 30.1 |
| 5 | 0.39 | 8.8 | 9740 | 16.1 |
| 6 (youngest) | 0.09 | 2.0 | 5090 | 8.4 |
| stem (epicotyl) | 1.56 | | 20790 | |
| main root | 0.48 | | 8000 | |
| lateral roots | 0.33 | | 68110 | |

each plant part the activities present in the ethanol-water soluble, the chloroform soluble, and the ethanol-chloroform insoluble substances were determined. The distribution of the radioactivity among the successive leaves is presented in Table 1. Leaf number 1 represents the oldest leaf present, leaf number 6 the three or four very small top leaves, which were not separately analysed. Table 1 clearly shows that the largest leaves also received the largest amounts of carbon-14. If, however, the specific activities of the various leaves are compared (Fig. 1), it is evident that these values were highest in the top leaves and decreased with increasing leaf age.

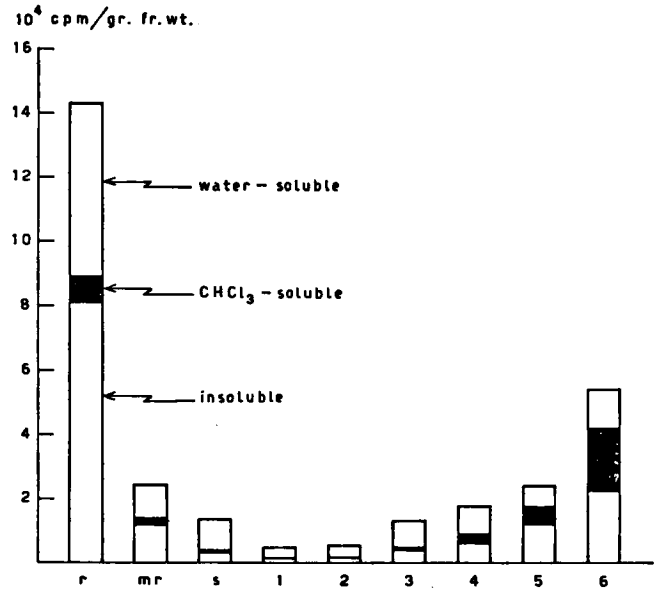


Fig. 1. The specific activities of various parts of a young tomato plant, 4 hours after the supply of $^{14}C$ (U) L-glutamic acid to the root system. r, lateral roots; mr, main root; s, stem; 1–6, leaves (oldest-youngest).

*The chemical conversions of the absorbed glutamic acid*

The more detailed analyses show that the youngest leaves contained relatively large amounts of labelled insoluble and chloroform-soluble compounds. They also show that the percentages of total leaf activity localized in both these groups of substances declined with increasing age of the leaves. Simultaneously the percentage of activity present in the ethanol-water soluble form increased (Fig. 2). These results suggest the existence of an inverse relationship between the degree of maturity of the leaf and the rate of incorporation of the labelled amino acids into proteins and lipoids.

Paper chromatography and subsequent autoradiography revealed about 12 radioactive substances in the ethanol-water soluble fractions of the various plant parts. Glutamic acid, $\gamma$-amino butyric acid and glutamine were the main amino acids present. Another quantitatively important active compound always present was malic acid. Small amounts or traces of activity were detected in aspartic acid, citric acid, succinic acid, $\alpha$-ketoglutaric acid and a few unknown organic acids. Hardly any, or no activity at all, was observed in proline, arginine, lysine, serine, glycine, $\alpha$-alanine, the leucines, valine and threonine.

In the largest leaves glutamic acid accounted for 50 per cent or more of all amino acid activity. In the youngest leaves $\gamma$-aminobutyric acid was the main active compound. Table 2 shows the distribution

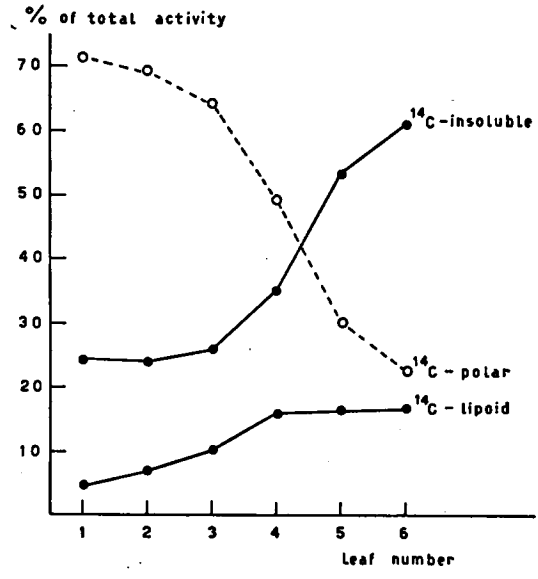


Fig. 2. The distribution of Carbon-14 among the lipoid (chloroform soluble), the "polar" (ethanol-water soluble), and the insoluble fractions of a young tomato plant, expressed as percentages of total leaf activity.

TABLE 2

The distribution of activity among the four active amino acids of leaves, bleeding sap and roots following uptake of $^{14}C$-L-glutamic acid.

| | Glu | $\gamma$ AB | Glu-$NH_2$ | Asp |
|---|---|---|---|---|
| Leaf no. 6 . . . . | 32 % | 60 % | 2 % | 6 % |
| ,, ,, 5 . . . . | 42 % | 45 % | 10 % | 3 % |
| ,, ,, 4 . . . . | 58 % | 40 % | 1 % | 1 % |
| ,, ,, 3 . . . . | 48 % | 45 % | 4 % | 3 % |
| Bleeding sap . . . | 10 % | 52 % | 36 % | 2 % |
| Roots . . . . . | 28 % | 50 % | 20 % | 2 % |

of the $^{14}C$ among the 4 active amino acids present in the various leaves, in comparison with roots, and with bleeding sap, that was obtained in a parallel experiment.

*Protein synthesis in the roots*

Isolated root systems were allowed to bleed on the $^{14}C$-glutamate solution described above. After 4 hours, comparable amounts of the amino acids from bleeding sap, the ethanol soluble fraction of the roots, and the root proteins were analysed by paper chromatography. Table 3 distinctly shows that all amino acids present in the protein hydrolysate were labelled, while no measurable amounts of activity could be detected in the corresponding free amino acids of the roots and the xylem exudate (bleeding sap).

TABLE 3

The activities of free and protein-bound amino acids of roots and of the free amino acids of bleeding sap, as measured by direct counting on the chromatograms, expressed in counts per minute per spot. —, less than 2 cpm.

| | Proteins | Free amino-acids | Bleeding sap |
|---|---|---|---|
| γ-amino butyric acid | — | 712 | 61 |
| glutamine | — | 1628 | 160 |
| glutamic acid | 144 | 336 | 72 |
| aspartic acid | 50 | 24 | 5 |
| α-alanine | 51 | — | — |
| arginine | 37 | — | — |
| lysine | 27 | — | — |
| proline | 34 | — | — |
| leucines | 69 | — | — |
| serine glycine | 15 | — | — |
| valine | 16 | — | — |
| threonine | 19 | — | — |

As the experiments of the present study were not designed to study protein synthesis, it was not possible to distinguish between net synthesis of proteins and what is commonly called protein turnover. Nevertheless the results show that all protein amino acids can be synthesized from supplied glutamate and may be mainly or exclusively incorporated into proteins.

*The influence of the root system on the distribution of the labelled substances*

In the experiments described so far the glutamic acid was supplied to the roots of intact plants. Further experiments were designed in an attempt to demonstrate possible direct influences of the root system on the distribution of $^{14}C$ among the successive leaves of the plant. Three groups of plants were used:

(a) The root system was cut off, and the glutamic acid solution was supplied to the basal part of the shoot.
(b) The glutamic acid was supplied to the root system of intact plants.
(c) The solution was supplied to root systems of de-topped plants. The exuding bleeding sap was collected.

The solutions used contained 1 mg of $^{14}C$ (U) L-glutamic acid (10 μc) in 10 ml of water. After 4 hours the experiments were stopped and the plants and the exudates were analysed.

Important differences in the distribution of the label in the plants of group (*a*) and (*b*) could not be detected (Fig. 3 and 4). Most of the radioactivity was present in the largest leaves, but if expressed per unit fresh weight, the youngest leaves of both groups of plants contained the largest amount of Carbon-14.

If the results shown in Fig. 3 and 4 are expressed in the way of Fig. 1, three similar patterns are obtained. The percentages of the insoluble, chloroform soluble, and ethanol-water soluble substances in the successive leaves of both groups of plants also showed a similar

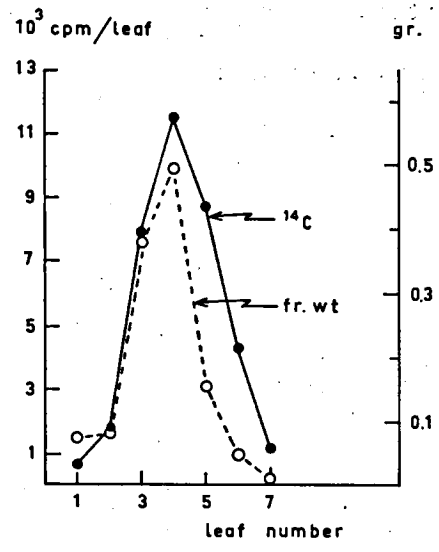


Fig. 3. Carbon-14 contents and fresh weights of the leaves of a young tomato plant, 4 hours after $^{14}C$ (U) L-glutamic acid was supplied to the root system.

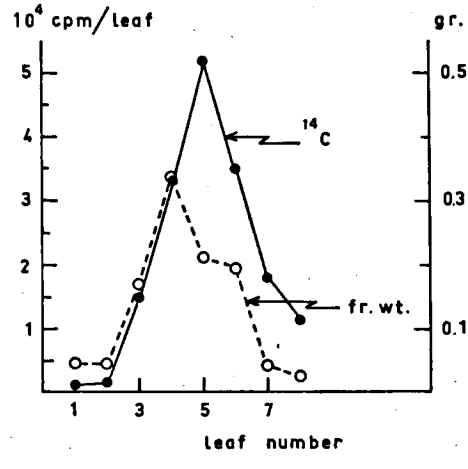


Fig. 4. Carbon-14 contents and fresh weights of the leaves of an excised tomato shoot, 4 hours after $^{14}C$ (U) L-glutamic acid was supplied to the cut stem base.

picture as that shown in Fig. 2. This was also the case for the qualitative and quantitative composition of the labelled amino acids from the various leaves (Fig. 5 and 6).

From the results obtained it is evident that the plants of groups (*a*) and (*b*) behaved similarly towards the supplied glutamic acid. Consequently a direct influence of the root system on the distribution

of the $^{14}C$ among the various aerial parts of the plant did not exist.

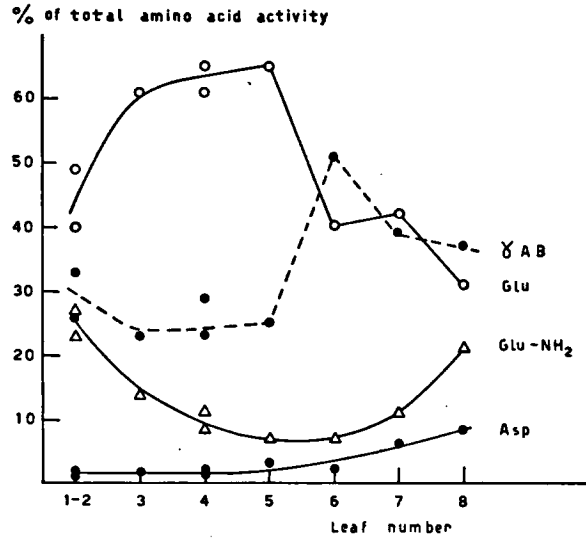


Fig. 5. Distribution of radioactivity among the free amino acids of the leaves of an intact tomato plant, 4 hours after the supply of $^{14}C$ (U) L-glutamic acid to the root system.

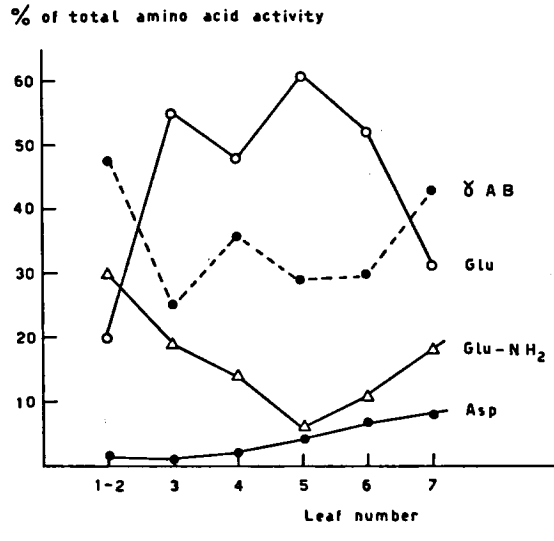


Fig. 6. Distribution of radioactivity among the free amino acids of the leaves of an excised tomato shoot, 4 hours after the supply of $^{14}C$ (U) L-glutamic acid to the cut stem base.

*Transpiration experiments*

A few experiments were carried out to determine whether differences in transpiration rate per unit weight of the successive leaves may have been responsible for the observed pattern of distribution of radioactivity. From a tomato plant the leaves were separately removed, directly weighed, brought into a constant stream of air for exactly 5 minutes, and re-weighed. The losses of weight were expressed as percentages of the original leaf weights. The transpiration-rate curve obtained (Fig. 7) shows a maximum for the largest leaves; the youngest and the oldest leaves have relatively low transpiration rates per unit weight. Consequently, the high specific activities of the young leaves can in no way be explained on a basis of a simple passive translocation by means of the transpiration stream.

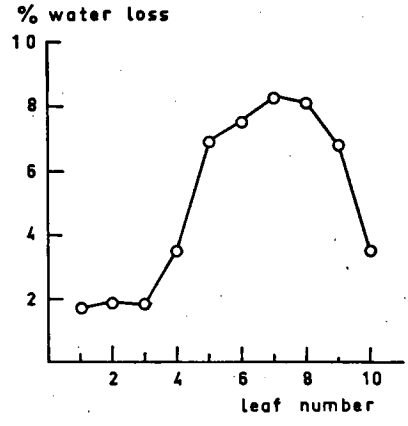


Fig. 7. The relation between leaf number and water loss from excised leaves after 5 minutes of transpiration in a stream of air at 20° C. Water losses are expressed in percentages of the original fresh weight of the successive leaves. Leaves no. 1 and 2 are the cotyledons.

## Discussion

The uptake of inorganic ions and water by tomato roots and their transport across the root tissues to the xylem is believed to involve both passive and active processes: Mass movement in the transpiration stream and an active mechanism of translocation responsible for their movement into the xylem of slowly transpiring, guttating, or bleeding plants [Van Andel, 1953; Lopushinsky and Kramer, 1961; Jensen, 1962]. A similar distinction can be made for the movement of glutamate across tomato roots. Its active uptake by tomato roots probably occurs by carrier systems, just as has been postulated for inorganic ions, and for amino acid uptake by carrot tissue [Birt and Hird, 1958], wheat roots [Wright, 1961] and micro-organisms [Britten and McClure, 1962].

The results of the present glutamate absorption experiments show that the largest part of the label exported by the roots has moved

into the largest leaves. This apparently points to an important role of the transpiration stream in the translocation of these substances. The youngest leaves, however, obtained the highest specific activity, i.e. the activity expressed per unit weight or unit area. The specific activity steadily declined with increasing degree of maturity of the leaves, a phenomenon that cannot be explained by transpiration rates only. Transpiration rates, however, do not account for all the water that moves into expanding leaves. By their much higher proportions of hydrophilic colloids, meristematic tissues are able to develop higher diffusion pressure deficits than older tissues. Tomato stem tips continue to obtain water during the daylight hours, while the rest of the stem is loosing it [WILSON, 1948]. In tobacco even under conditions of severe wilting of the leaves, water and nitrogenous substances move into the meristematic regions of the stem tip in quantities sufficient to permit a continuation of growth [MOTHES, 1931]. Many reports, however, distinctly show a translocation of nitrogenous materials that in no way can be explained by a passive mass flow. The data on the transport of soluble nitrogenous substances to developing seeds of maize, barley and tobacco are very convincing in this respect [for literature, e.g. MCKEE, 1962].

The chemical analyses demonstrated that the percentages of the insoluble and chloroform soluble labelled substances—mainly proteins and lipids—declined with increasing age of the leaves, while the percentages of the labelled ethanol-water soluble substances simultaneously increased. A similar phenomenon has been reported by ARONOFF [1962] for soya leaves: The majority of the active amino acids synthesized from $^{14}CO_2$ appeared to occur in the proteins and a trace in the amino-acid pool of the young leaves. In mature leaves, however, the reversed situation was found. Results of BELIKOV [1961] point in essentially the same direction.

The experiments with plants with and without roots distinctly showed that the distribution of the amino acids among the leaves is regulated by the aerial parts of the plant. The roots do not have any *direct* influence on the ultimate fate of the amino acids exported by them. The present experiments, however, do not exclude more *indirect* regulating activities of the roots, for example by the synthesis of kinetin-like substances, which may be translocated to the shoot [KULAYEVA, 1962] and act on the ribonucleic acid and protein metabolism of the leaves [JENSEN and POLLOCK, 1958; MOTHES, ENGELBRECHT and KULAYEVA, 1959].

The paper chromatograms of the ethanol-water soluble fractions revealed the presence of various direct conversion products of glutamic acid, but other $^{14}C$-labelled amino acids, as proline, $\alpha$-alanine, glycine, serine, arginine, lysine, valine, (iso)leucine, could not be detected in their free state. It was quite surprising, therefore, that all these $^{14}C$-amino acids were found in the protein hydrolysate of the roots. Consequently, the roots synthesize all protein amino acids from supplied glutamate, but apparently these substances do not mingle freely with the amino acids, that are stored in the free state in the

cells. Probably they are formed close at the sites of protein synthesis [cf. STEWARD and BIDWELL, 1962]. Only those amino acids, which are synthesized in relatively large amounts from the glutamate or are not used in protein synthesis (e.g. $\gamma$-amino butyric acid) enter the xylem vessels or the pools of free amino acids. The protein amino acids synthesized from the glutamate and incorporated into the root proteins are consequently selectively withdrawn from the stream of glutamate and its conversion products that moves across the roots. Nevertheless, in the long run, protein turnover will cause the appearance of these protein amino acids in the amino acid pools and in the xylem exudate, as both the non-protein and protein amino acids move through the xylem in an upward direction. The latter, however, probably have a protein origin.

The rate of translocation of the various conversion products of glutamic acid to the xylem vessels will mainly depend on the rate of water movement across the root tissues. Their proportions in the xylem will be relatively high if glutamate and water movement through the root tissues is slow, as in bleeding (Table 2 and 3). With high transpiration rates, however, glutamate itself will be the main component in the transpiration stream and so in the amino acid fractions of the large leaves. During and after their translocation through the xylem vessels, amino-acid uptake takes place, presumably by empty carriers present in the surrounding symplasm. Intense net synthesis of proteins in a tissue will favour de-loading of the carriers and, by that, favour the translocation of amino acids in its direction.

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