

The tissue samples were weighed and stored at -20°C until used. Ethanol extraction of the tissue and microprocedures (based on fluorometric measurements and enzymic methods) for the assay of GABA were carried out according to Graham *et al.* (7); glycine and total amino acids were determined by the procedures described by Graham *et al.* (2).

We observed a significant decrease of glycine in the grey matter on the side of local tetanus ($t = 4.72$, $P < .005$); changes in concentration in the white matter on either the control or local tetanus side were not significant. Concentrations of GABA and total amino acids in both grey and white matter on the side of local tetanus did not differ significantly from those found on the control side (Table 1).

Glycine and GABA are viable candidates for an inhibitory transmitter in the mammalian central nervous system. Glycine may be considered a likely inhibitory transmitter in cat spinal cord, by virtue of demonstration of association between glycine and lumbosacral interneurons (3), and by electrophysiological analysis of the effect of glycine on spinal motoneurons (4). Tetanus rigidity has been explained as the result of blockade of inhibitory transmission to the α -motoneuron of cat spinal cord (6) and to the γ -motoneuron (8).

Our interest is focused on the inhibitory transmitter in the spinal cord of cats with tetanus rigidity. Our results indicate an association between loss of glycine and presence of local tetanus rigidity, while GABA concentration did not change, and are consistent with findings on anoxic rigidity (3). These together suggest that glycine acts as an inhibitory transmitter in cat spinal cord and that tetanus toxin blocks spinal inhibitory transmission by decreasing a transmitter. γ -Aminobutyric acid does not appear to be an inhibitory transmitter in cat spinal cord.

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Translocation in Perennial Monocotyledons

Abstract. *Gross autoradiography, historadiography, and electron microscopy provide evidence that enucleate sieve elements in basal internodes of relatively old Tradescantia plants are still functional.*

Commonly, the sieve elements of angiosperms are characterized as short-lived cells, presumably functioning in the conduction of assimilates for only one season. *Tilia*, the linden tree, has often been cited as an exception—the sieve elements of *T. americana* living, and presumably remaining functional, for as many as 5 years (1); those of *T. cordata* living for as many as 10 years (2). The concept of short longevity of sieve elements in angiosperms comes mainly from investigations on deciduous dicotyledons. Until recently the sieve elements of the perennial monocotyledons seemed to have been forgotten. Yet it would seem reasonable to assume that in this group of plants, most of which lack secondary tissues, some sieve elements function for many years or for the life of the plant parts in which they occur.

It was not surprising, therefore, that Ervin (3) found living sieve elements in 8-year-old stem segments of *Smilax hispida* and in 10-year-old rhizome segments of *Polygonatum canaliculatum*, or that Parthasarathy and Tomlinson (4) demonstrated living sieve tubes at the base of a *Sabal palmetto* stem at least 50 years old. Tomlinson (5) estimates that in some of the slower-growing arborescent monocotyledons the age of the conducting tissues must exceed a century.

The mature, presumably functional, sieve element of angiosperms is generally described as an enucleate, tonoplast-free cell. As the sieve element approaches maturity its nucleus and tonoplast (the membrane separating vacuolar contents from cytoplasm) disappear, and the contents of adjacent sieve elements become continuous through newly formed pores in their common walls. Recently, it was demonstrated that aphids were able to feed

on mature sieve elements of secondary phloem in their 2nd year in *T. americana*, evidence that such sieve elements were still functional (6). No such evidence is available to show that sieve elements of the oldest part of the primary phloem of monocotyledons are able to conduct, for aphids do not feed on old stems of monocotyledons. Therefore, a monocotyledonous species

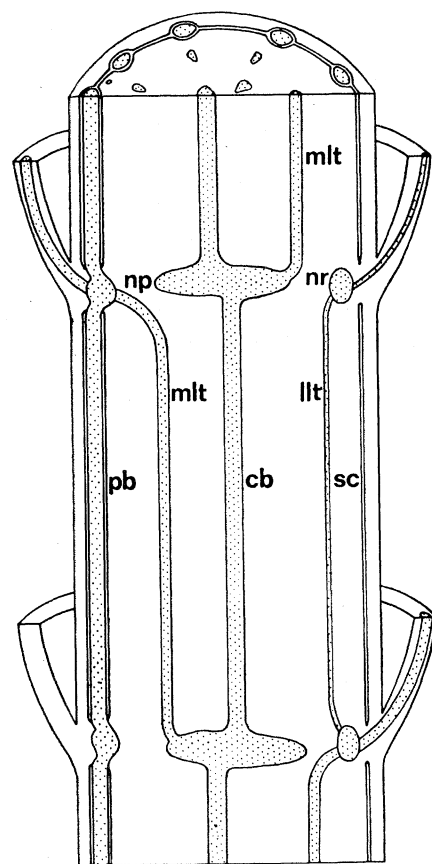


Fig. 1. Arrangement and relationships of the vascular bundles (stippled) in the stem of *Tradescantia albiflora*. Abbreviations: *cb*, central bundle; *llt*, lateral leaf trace; *mlt*, median leaf trace; *np*, nodal plate; *nr*, nodal ring; *pb*, peripheral bundle; and *sc*, sclerified endodermis.

had to be found which was small enough for translocation experiments with a mobile radioactive tracer in the phloem and also suitable for gross autoradiography and historadiography. *Tradescantia albiflora*, a fast-growing perennial monocotyledon, fulfills these requirements.

Cuttings of *T. albiflora* were cultivated in both pots and nutrient solution for up to 30 months. Adventitious roots associated with branches were prevented from coming in contact with the soil. The original roots were the only pathway for water and minerals, and the assimilates used for root growth had to traverse the basal internode.

The stem (Fig. 1) of *T. albiflora* contains four types of vascular bundles. Three of them (*cb*, *llt*, and *mlt*) are situated inside the endodermis and are, without exception, leaf traces. They have no metaxylem. The fourth type of bundle (*pb*) differentiates relatively late, produces metaphloem and metaxylem, and is surrounded by endodermal cells, the walls of which eventually become lignified. The bundles of the fourth type are axial and are connected in the nodal rings (*nr*) with leaf traces (*llt* and *mlt*).

For tracer experiments, DL-phenylalanine-1- C^{14} was used. Its L-isomer was found to be extensively incorporated into the roots (7). A few days before application, the nutrient solution was aerated to stimulate root growth. The tracer was applied to an exporting leaf of a basal branch. After 5 or more hours of incubation the whole plant was quickly frozen with crushed dry ice and lyophilized; it was spread on a sheet of paper and then exposed to x-ray film. The basal leaves of the main shoot of a plant cultivated for 15 months before application of the tracer (Fig. 2A) had dried and dropped long before the experiment started. The lateral shoot is intensively labeled. The main shoot (Fig. 2B) below the node of insertion of the lateral shoot shows two black lines which represent two peripherally located bundles. The roots are heavily labeled, but the shoot above the node of insertion appears white, except for some sites (arrows) on lateral shoots which seem to have accumulated the tracer.

Small pieces of the basal internodes were sawed out of a similarly treated 15-month-old plant while it was frozen. These stem pieces were lyophilized and embedded in paraplast according to the method of Eschrich (8). Cross sec-

tions, 10 μ m in thickness, served for historadiographs. The procedure followed has been described elsewhere (8, 9).

Examination of the processed slides revealed two peripheral bundles dis-

tinctly labeled in the phloem moiety. Figure 2 also shows one of the bundles in the plane of the section (Fig. 2C) and in the plane of the photoemulsion (Fig. 2D). Since the metaphloem consists only of sieve tubes (*st*) and companion

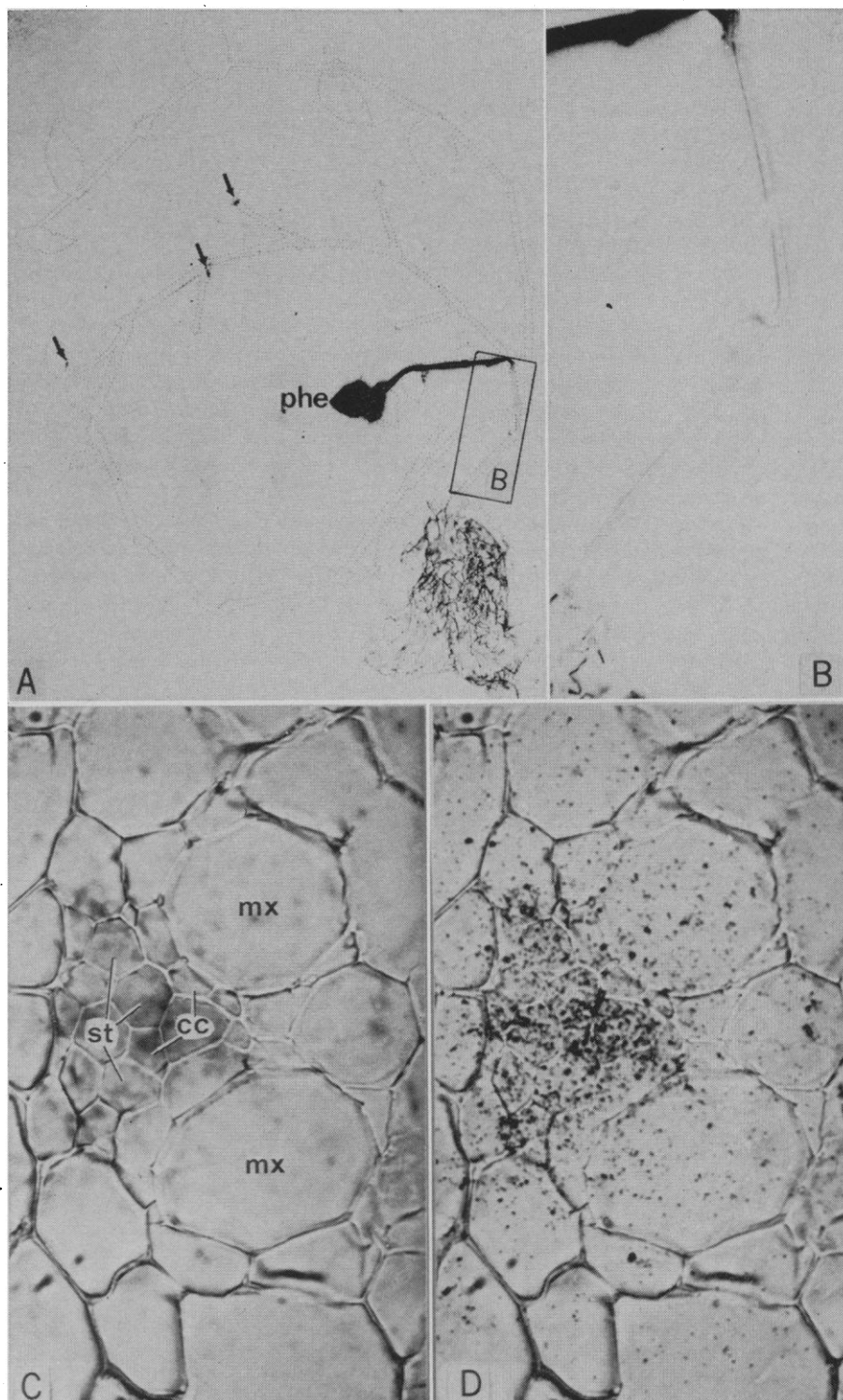


Fig. 2. (A) Autoradiographs of basal part of 15-month-old plant, a leaf of which was treated for 5 hours with phenylalanine-1- C^{14} . The tracer moved mainly to the roots. A small amount was accumulated at sites of some lateral shoots (arrows). (B) Basal internodes of same plant with two dark lines representing tracer-conducting peripheral bundles. (C and D) Historadiograph (D) of a cross section (C) of a peripheral bundle similar to those shown in B ($\times 400$); *cc*, companion cells; *mx*, metaxylem vessels whose secondary walls have been reduced by lyophilization; and *st*, sieve tubes.

cells (cc), it is safe to assume that these cells, probably only the sieve tubes, were involved in translocation of the tracer. The two labeled bundles of the cross sections obviously correspond to the two dark lines of the internode of Fig. 2B. These bundles were connected with the branch traces of the lateral shoot, and the branch traces were connected with the median trace of the treated leaf.

Portions of basal internodes of 15- to 26-month-old plants were fixed in a mixture of osmic and chromic acids for electron microscopy. Generally, sieve elements of the peripheral bundles appeared empty. The wall of the sieve element was lined with a plasma-lemma and remnants of cytoplasmic membranes, possibly endoplasmic reticulum. Occasional small mitochondria and plastids with dense triangular inclusions, apparently typical of monocotyledonous sieve elements (10), were associated with the parietal membranes. The cell lumen contained a filamentous substance which appeared distorted or disrupted. A similar substance in *Elodea* sieve elements has been interpreted as slime (11). All sieve elements examined lacked nuclei. The sieve-area pores were lined with callose and occluded with dense masses of filamentous material. The distorted appearance and uneven distribution of the filamentous substance within the sieve elements, plus the presence of numerous inclusions free of the plastid membranes, suggest that the contents had undergone considerable deformation during manipulation of the tissue. The nucleate companion cells contained numerous mitochondria and vacuoles and were relatively undisturbed when compared with the apparently highly disturbed sieve-element contents. This difference emphasizes that intact sieve tubes probably have high turgor pressures.

Results of the present investigation provide evidence that at least certain sieve tubes of the primary phloem of perennial monocotyledons remain functional for long periods of time or as long as the plant part in which they occur remains alive.

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Lysergic Acid Diethylamide:

Effects on the Developing Mouse Lens

Abstract. High doses (5×10^{-6} gram) of LSD-25 given to Swiss-Webster females on gestation days 6, 7, 8, or 9 caused a high incidence of anterior subcapsular lens abnormalities. Accompanying this, the lens epithelium was often hyperplastic, and the lens bow was widened posteriorly in a fashion similar to cataracts induced by x-radiation. Confirmation of this effect of LSD-25 was obtained by a (duplicate) experiment 1 year after the observations reported.

The teratogenic effects of LSD-25 on the fetuses of the rat (1), mouse (2), and hamster (3) have been reported, with results varying from a decrease in litter size (1) to malformations of the central nervous system (2, 3). We now report the effects of LSD-25 on the developing lens. The abnormality has heretofore not been described; however, certain aspects of it are similar to lens changes induced by x-rays in mice (5-7), rats (5, 7), and rabbits (4, 5), and to anterior ocular inflammation in man (8).

Pregnant Swiss-Webster mice in groups of six were given single interperitoneal doses of 5×10^{-6} g of LSD-25 on gestation days 6, 7, 8, or 9; embryos were killed on day 18. The equivalent human hallucinogenic dose would

be about 0.17 μ g per 25 g of body weight. Our dose of 5 μ g was 30 times this amount. Because we did not know the effects of high doses of LSD-25 on fetal survival, embryos were removed on gestation day 18 to check for resorptions. Control animals were in two groups: the first consisted of subgroups of five mice treated with 1×10^{-6} of sodium potassium tartrate (the carrier of LSD-25) on days 6, 7, 8, or 9, and the second consisted of eight pregnant females not treated. All embryos were killed on gestation day 18, and whole heads were fixed in cold Carnoy's solution for at least 72 hours. After the whole heads were dehydrated in alcohol of increasing concentration up to 100 percent, they were then placed in methyl benzoate and benzene,

Table 1. Percentages of abnormal lenses in fetuses of LSD-treated mice.

Gestation age	Females* (No.)	Fetuses (No.)	Eyes examined (No.)†	Abnormal/total	Percentage of abnormal lenses
<i>Experimental group—LSD-25</i>					
6	3	13	26	21/26	81
7	5	54	99	64/99	65
8	5	21	31	17/31	55
9	5	32	61	48/61	79
<i>Control group—treated</i>					
6	4	34	32	0/32	
7	5	41	53	0/53	
8	6	61	71	0/71	
9	5	44	62	0/62	
<i>Control group—untreated</i>					
	8	61	122	0/122	

* The discrepancy in the number of animals treated in each group and the number listed in this table is due to nonpregnant animals. † The disparity between the number of fetuses and number of eyes examined is due to difficulties in processing.