

Sites of Accumulation in Excised Phloem and Vascular Tissues

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Summary. Excised pieces of vascular bundle and phloem tissue were allowed to accumulate radioactive phosphate and sulfate, and were then sectioned and autoradiographed so as to detect the sites of accumulation. Special methods were needed to prevent any diffusion of the radioisotope. Some autoradiographs obtained are presented. In excised celery vascular bundles, the most radioactive area and hence the most actively accumulating tissue was the young secondary phloem at the sides of the bundle. In intact plants, the same tissue was the most active in translocating. In excised apple phloem there was some variation in behavior, but again the young secondary phloem was generally the most actively accumulating tissue. Accumulation activities of individual cells in the phloem and vascular tissue were compared. It appeared that all cell types, ray, phloem and xylem parenchyma, cambial cells and sieve tubes, accumulated at least 5 times more actively than did the cortical parenchyma cells. The sieve tubes were among the most actively accumulating cells present, accumulating 20 times more actively than the cortical parenchyma cells. It is concluded that accumulation processes have a primary role to play in the mechanism of phloem transport.

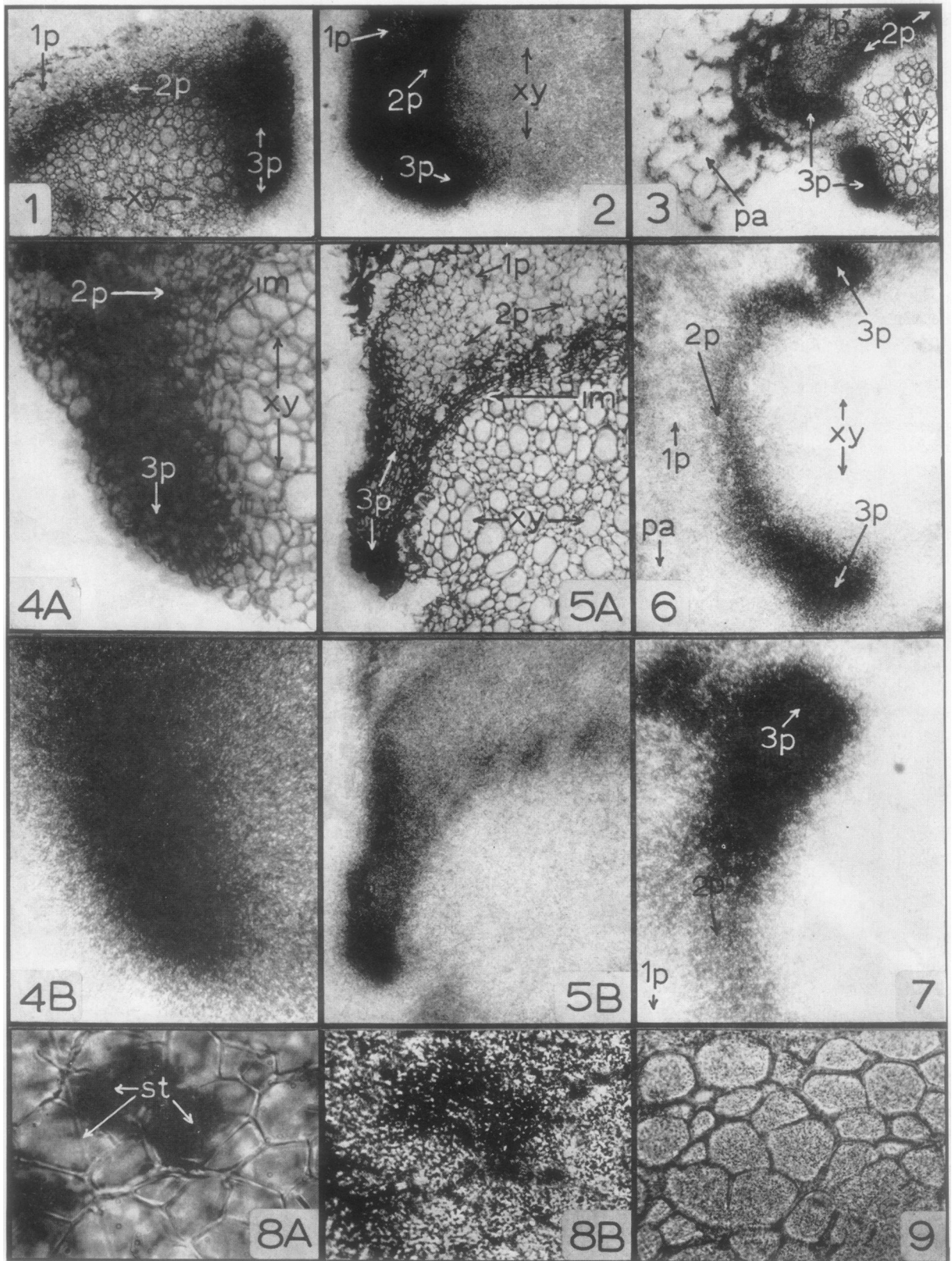
In the preceding paper (2) it has been demonstrated that excised phloem and vascular tissues take up solutes from external solutions by a true accumulative process, under metabolic control, and against high concentration ratios. Phosphate, sulfate and sucrose were each accumulated up to 40 times faster than by comparable parenchyma tissues; and sucrose taken into the tissue stayed mainly in the form of sucrose, the major translocatory carbohydrate. These results suggested that accumulation mechanisms were involved somehow in the phloem translocation process of the intact plant: to elucidate the role they might play, we require a knowledge of the sites of accumulation in the excised tissues. Should accumulation occur solely into phloem parenchyma cells for example, the role would probably be secondary, since most translocation is believed to take place via the sieve tubes. However, if any considerable amount of the solute was being accumulated into the sieve tubes themselves, there would be good grounds for considering that anion and sugar accumulation processes could have a primary role to play in the phloem translocation process.

An attempt has therefore been made to identify, by autoradiography, the cellular sites of accumulation in excised phloem and vascular tissues. Most of the accumulated material was water-soluble, and so special sectioning and autoradiographic techniques had to be developed so as to preserve cellular detail without causing redistribution of the radioactive material. Difficulties were such that experiments employing sucrose- ^{14}C would have been too expensive, and so the accumulation sites of phosphate and sulfate alone were studied.

Methods

Preparation of Excised Tissues and Accumulation of Radioactive Anions. Apple phloem and celery vascular bundle tissues were isolated as described previously (2), and washed in 4 changes of 10^{-4} M CaSO_4 (for phosphate- ^{32}P experiments) or 10^{-4} M CaCl_2 (sulfate- ^{35}S experiments) for 16 hours at 24° on a rotary shaker. The subsequent experimental schedule was designed to minimize any tendency of the anions to adsorb nonspecifically to the tissue. Tissues were aerated on a rotary shaker at 24° in A) distilled water, 15 minutes; B) nonradioactive 10^{-4} M KH_2PO_4 (phosphate accumulation experiments) or 10^{-4} M K_2SO_4 (sulfate accumulation experiments), 15 minutes, in order to fill adsorption sites with non-radioactive anion; C) distilled water, 3 changes, 45 minutes; D) 10^{-5} M $\text{KH}_2^{32}\text{PO}_4$, 10 $\mu\text{C}/\text{ml}$ or 10^{-5} M $\text{K}_2^{35}\text{SO}_4$, 40 $\mu\text{C}/\text{ml}$, for 4 hours; E) distilled water, 4 changes, 15 minutes; F) nonradioactive 10^{-4} M KH_2PO_4 or 10^{-4} M K_2SO_4 , 15 minutes to remove exchangeably adsorbed radioactive material; G) distilled water, 20 minutes. Tissues were then blotted gently and quick-frozen in toluene at -78° (dry ice/ethanol bath).

Translocation in Intact Celery Petioles. To contrast the behavior of excised bundles with that of bundles present in the intact plant, the site of translocation in intact celery leaves was studied. Mature growing celery plants were used. The radioactive solutions (10^{-4} M $\text{KH}_2^{32}\text{PO}_4$, approx 2 mc in 0.2 ml per leaf; and 10^{-4} M $\text{K}_2^{35}\text{SO}_4$, approx 3 mc in 0.4 ml per leaf) were injected into the hollow center of the subsidiary petiole of 1 leaflet, or (plus 0.5% Tween



20 detergent and 5% sucrose) painted onto the leaf surfaces. After 1 or 4 hours, the leaflets and subsidiary petioles were cut off and discarded; then short lengths of the main petiole were cut, and segments containing bundles were excised and immediately quick-frozen.

Drying, Embedding and Sectioning Tissue. To avoid any redistribution of the water soluble materials, each step was carried out in such a manner that solute diffusion could not take place. Frozen tissue was removed from the toluene, placed on a pre-chilled filter paper pad on a block of dry ice, and covered with powdered dry ice, so as to allow the toluene to drain off; then transferred to a vacuum desiccator prechilled to -30° , containing P_2O_5 as desiccant. The desiccator was evacuated to 0.1 mm Hg and held there at -25° for 1 week, then brought to room temperature. The vacuum was then slowly released, during 5 minutes, through a P_2O_5 trap. Tissue was transferred quickly, so as not to pick up traces of atmospheric moisture, to a tube containing molten embedding wax, and a metal gauze shield was thrust down to submerge the tissue. The tube was held at 60° and evacuated to 0.05 mm Hg for 2 hours, then the vacuum was released through a very slow leak over the next 10 hours. Pieces of tissue were then embedded in blocks in the conventional way.

Sections were cut at 5, 10 and 20 μ , in transverse section (TS), radial longitudinal section (RLS) and tangential longitudinal section (TLS), on a Cambridge microtome, on nonhumid days. Ribbons were either gathered on a large rubber bung that had previously been dusted with talcum then wiped clean, and mounted directly (by method I); or they were collected in vials which were kept over P_2O_5 until the sections were required for mounting (by methods II and III).

Mounting and Autoradiographing Sections. In method I, the paraffin ribbon containing the sections

was pressed onto the surface of the autoradiograph film, autoradiographed, then moistened to cause the sections to adhere to the film, and the film was then processed (10). In method II, sections were freed of paraffin wax, stained, mounted in canada balsam, and autoradiograph film was laid over the top, exposed and processed. In method III, sections were dewaxed and stained, then laid directly on the surface of the autoradiograph film, autoradiographed, moistened and the film was processed.

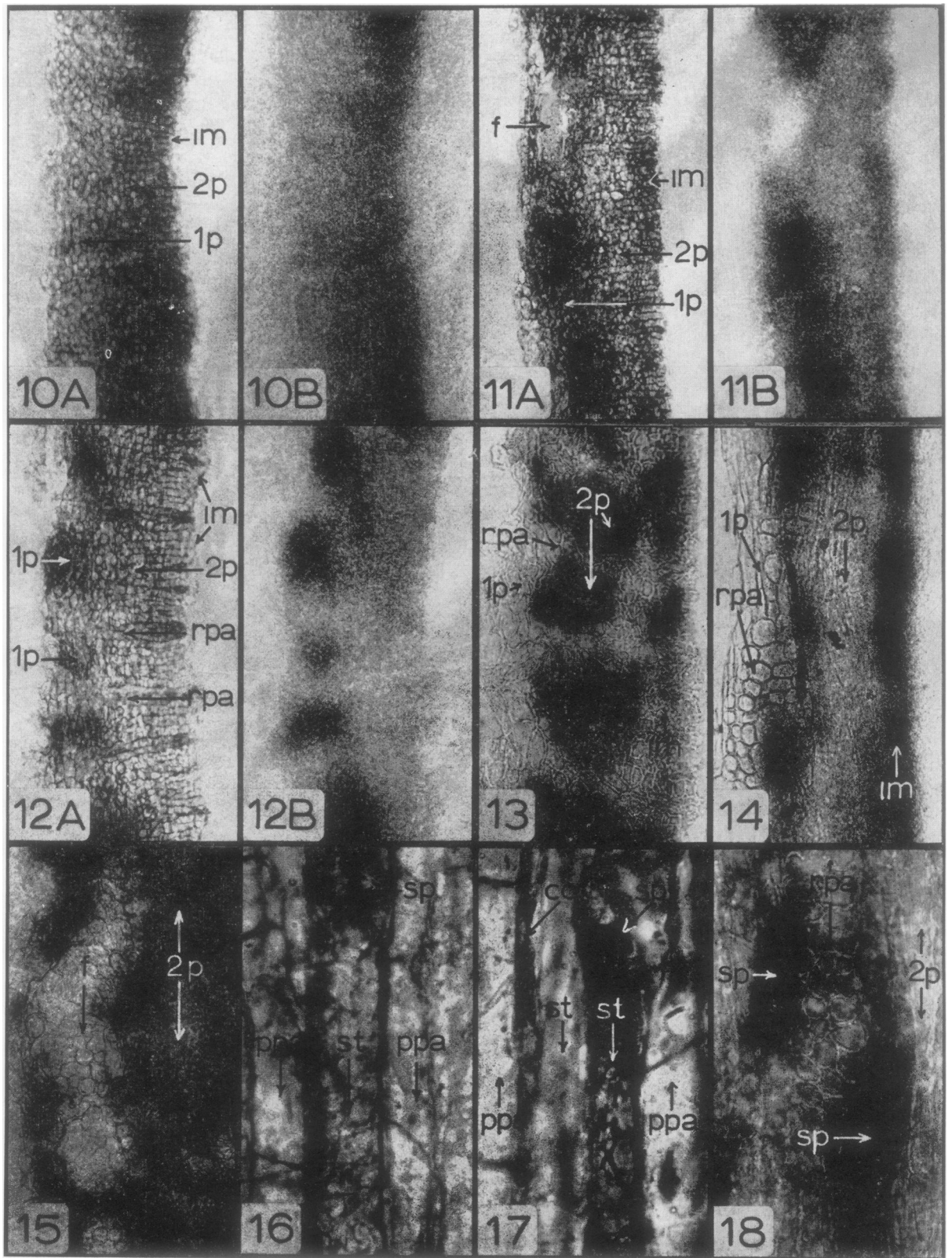
The detailed procedure for method I is as follows: Microscope slides were cleaned in alcoholic KOH, and then dipped in 0.5% gelatin + 0.05% chrome alum, drained and dried. In a darkroom, 4×5 cm pieces of Kodak AR 10 stripping film were prepared and floated, emulsion side up, on water at 20° . The pieces of film were collected, emulsion side out, on the coated glass slides, which were then dried in a warm air stream and stored in a light-tight cassette until required.

The paraffin ribbon on the rubber bung was warmed under an infrared lamp and eased with needles to allow it to stretch. In the darkroom a prepared slide was placed, emulsion side down, upon the ribbon, and then pressed to flatten and transfer the ribbon to the slide. Slides were held for 1 to 4 weeks over silica gel. Then in the darkroom, slides were briefly held over a steam bath to moisten the sections, dried, dipped in water, redried, passed through 3 washes of xylol to remove wax, dried again, developed in Kodak D 19 developer (8 min at 20°), fixed, washed and dried. The sections were finally mounted in 45% glycerol or in Euparal. The method gave excellent resolution of the autoradiograph. However, cells in the section were often difficult to identify, and the slides were not very suitable for photomicrography.

Method II of mounting and autoradiographing the sections was therefore developed. Xylol, 3 ml, was added to vials containing lengths of paraffin ribbon,

FIG. 1–31: abbreviations. cc, companion cell; f, primary phloem fibers; im, immature and cambial cells; 1p, primary phloem; 2p, older secondary phloem; 3p, young secondary phloem; pa, cortical parenchyma; ppa, phloem parenchyma; rpa, phloem ray parenchyma; sp, sieve plate; st, sieve tube; v, vessel; xpa, xylem parenchyma; xy, xylem; TS, transverse section; RLS, radial longitudinal section; TLS, tangential longitudinal section; I, II and III, section autoradiographs prepared by methods I, II and III.

FIG. 1. Distribution of accumulated phosphate- ^{32}P in excised celery vascular bundle tissue. II, TS, X 25. Focus on section. FIG. 2. Distribution of accumulated sulfate- ^{35}S in excised celery vascular bundle tissue. II, TS, X 25. Focus on autoradiograph. FIG. 3. Distribution of accumulated sulfate- ^{35}S in excised celery vascular bundle tissue, showing accumulation in attached cortical parenchyma. I, TS, X 25. FIG. 4. A, cell and B, silver image, showing details of distribution of accumulated phosphate- ^{32}P in the phloem wing of an excised celery vascular bundle. II, TS, X 50. FIG. 5. A, cell and B, silver image, showing details of distribution of accumulated sulfate- ^{35}S in the phloem wing of an excised celery vascular bundle. II, TS, X 55. FIG. 6. Distribution of sulfate- ^{35}S in a translocating vascular bundle from the petiole of a leaf fed sulfate- ^{35}S 4 hours previously. II, TS, X 30. FIG. 7. Details of distribution of translocated sulfate- ^{35}S in the phloem wing of a translocating bundle from the petiole of a leaf fed sulfate- ^{35}S 4 hours previously. II, TS, X 55. FIG. 8. A, cell and B, silver image, showing accumulation of sulfate- ^{35}S in sieve tubes of phloem of excised celery vascular bundles. Two sieve tubes have accumulated more actively than the surrounding cells. III, TS, X 180. FIG. 9. Accumulation of sulfate- ^{35}S in phloem cells of celery vascular bundles. All cells show a similar radioactivity. III, TS, X 95.



and swilled around to dissolve the wax; then the xylol was removed carefully with a Pasteur pipette, leaving the sections in the vial. A second xylol wash was given, and the sections were then either mounted directly, or else the sections were washed in absolute ethanol then stained for 30 minutes with 0.2% fast green in absolute ethanol-clove oil (6:4, v/v) and washed twice in absolute ethanol and twice in xylol. This staining operation did not cause any detectable redistribution of radioactive material.

Sections were mounted in the following way. Sections, floating in xylol, were transferred with a wide bore dropper to a clean microscope slide, and excess xylol was removed with filter paper; then a drop of thin canada balsam was added. Sections were arranged with a needle, and a 2-cm square of 10 μ thick polythene film (Dow Handi-wrap), which had been presoaked in xylol, was laid over the balsam to form a cover slip. The thin balsam was spread by stroking the surface of the polythene with a square of filter paper. The slide was set aside for a day to dry (polythene is permeable to xylol) then excess balsam was scraped off and the polythene trimmed where necessary with a razor blade. The slide was wiped with a pad soaked in warm 0.5% gelatin + 0.05% chrome alum, was dipped in fresh solution, and set aside to drain and dry.

The slide was coated with stripping film in the conventional manner (film floated emulsion side down, and lifted out with the microscope slide so as to cover the sections); though care was needed to settle the film tightly on the surface of the polythene. Slides were dried, held for 5 to 40 days, developed and fixed. The stripping film stuck sufficiently firmly to the polythene surface that in only about 1 in 6 slides was there any movement of the film from the polythene during development. Such slides could be detected by a wrinkling of the stripping film, and were discarded. Cell detail was usually well preserved.

Method III was intended to combine the advantages of methods I and II. Sections were washed and stained, as for method II. Then, in a darkroom,

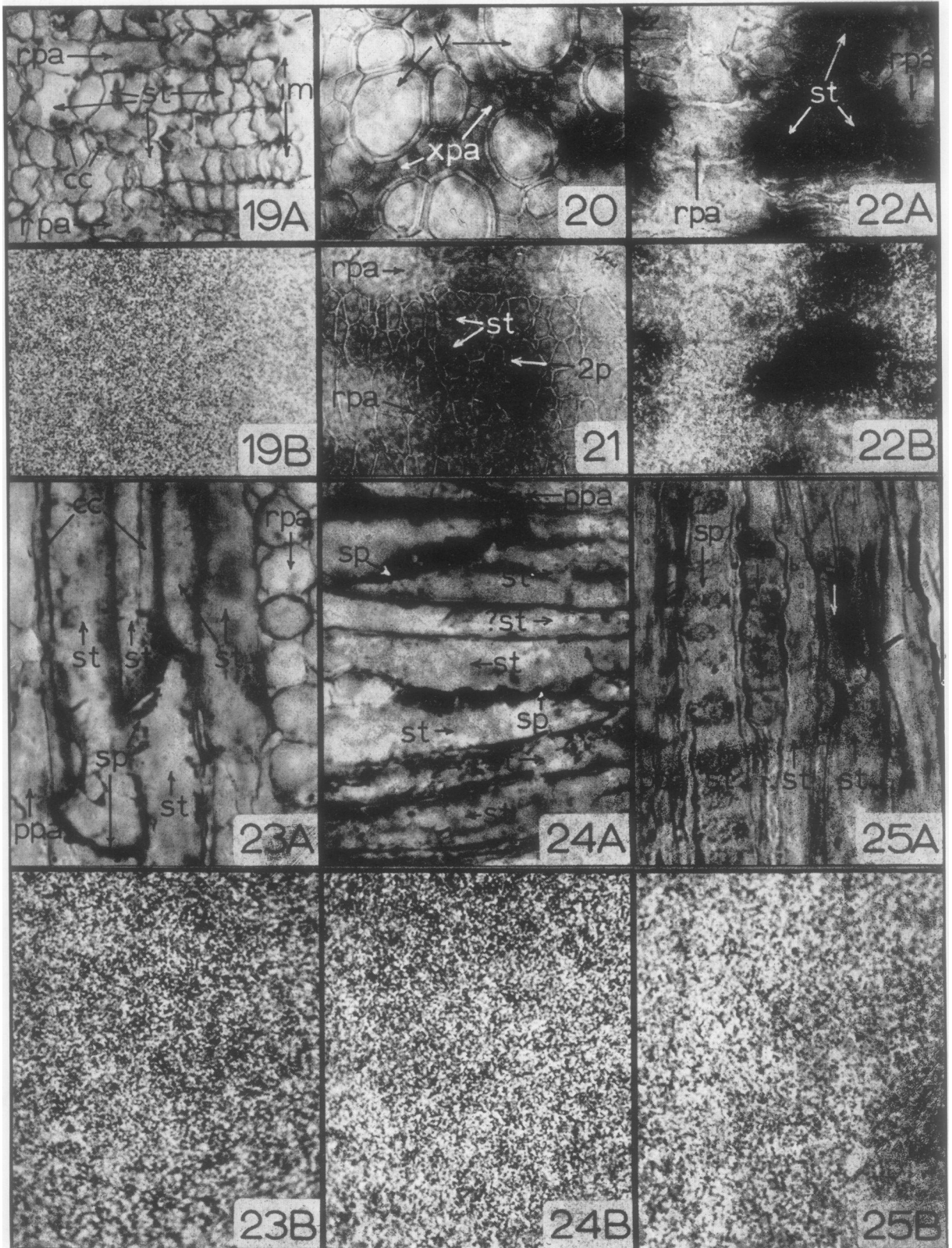
they were transferred, in a drop of xylol, on to the surface of microscope slides which had previously been covered with stripping film as in method I. The slides were dried, autoradiographed, and then processed as in method I. Sections were mounted in canada balsam under a cover slip. Only 20% of the sections remained in place during processing when this method was employed.

Photomicrography. Photomicrographs were taken with a Leitz photomicrograph attachment on Agfa IFF film. When the silver image of the autoradiograph was to be photographed, light for the microscope was passed through a Balzers Filtraflex K3 interference filter (430–530 $m\mu$) at a near-maximum condenser opening. Under these conditions, stained sections were almost invisible, and the image was due to the silver grains of the autoradiograph. When the stained section was to be photographed, light was passed through a K5 (575–700 $m\mu$) or K6 (620–710 $m\mu$) filter at a near-minimum condenser opening. OD of the section was then at a maximum; and with the focus on the section most of the image detail was due to the section, not the autoradiograph. Unstained sections were difficult to photograph, though visual observations were possible under phase contrast.

Results

Resolution of Detail. The resolution of the autoradiographs was estimated, from the sharpness of the autoradiograph at the margin where the radioactive section met the nonradioactive mounting medium (see fig 4, 5), by measuring the distance from the edge of the section at which the silver grain count of the autoradiograph had fallen to 20% of that over the section. With mounting methods I and III, resolution was approximately 5 μ for ^{35}S in 5 μ sections; 10 to 15 μ for ^{35}S in 10 μ and ^{32}P in 5 μ sections; and 25 μ for ^{32}P in 10 μ sections. These values agree with those reported by other workers (4, 11). Though this resolution was satisfactory, it was often difficult to identify the various types of cells. Sections mounted by method II, on the other hand, were

FIG. 10. A, cell and B, silver image, showing the general, rather patchy distribution of accumulated phosphate- ^{32}P in excised apple phloem tissue. In this section accumulation was more active in the young secondary phloem than in the primary and old secondary phloem. II, TS, X 30. FIG. 11. A, cell and B, silver image, showing the general, rather patchy distribution of accumulated sulfate- ^{35}S in excised apple phloem. In this section, accumulation was rather more active in the primary phloem than in the secondary phloem. The primary phloem fibers did not accumulate. II, TS, X 30. FIG. 12. A, cell and B, silver image, showing a marked concentration of accumulated sulfate- ^{35}S in the primary phloem of excised apple phloem tissue; and much lower amounts in the secondary phloem and the phloem rays. II, TS, X 30. FIG. 13. A marked concentration of accumulated sulfate- ^{35}S in the old secondary phloem cells of excised apple phloem tissue; and much smaller amounts in the primary and young secondary phloem, and in the phloem rays. I, TS, X 45. FIG. 14. A marked concentration of accumulated sulfate- ^{35}S in the young secondary phloem of excised apple phloem tissue. III, RLS (slightly oblique), X 50. FIG. 15. Showing the complete absence of accumulated sulfate- ^{35}S in the primary phloem fibers of excised apple phloem. I, TS, X 105. FIG. 16. Fibrillar structures in the lumen of a sieve tube. Note the presence of the structures along the length of the tube. II, TLS, X 190. FIG. 17. Fibrillar structures clustered near the sieve plate of a sieve tube. II, TLS, X 190. FIG. 18. A marked concentration of accumulated sulfate- ^{35}S over the sieve plate regions of 2 sieve tube pairs. I, TLS, X 90.



easily studied and photographed; but autoradiograph resolution was poorer, since the polythene covering layer resulted in a separation between the section surface and the emulsion surface. This separation was measured. With about 1 in 20 sections, the separation was only 11 to 14 μ , and with about 1 in 4 sections, 14 to 18 μ . The resolution of the resulting autoradiographs was 30 to 40 μ for ^{35}S and 100 to 125 μ for ^{32}P . A further 1 in 4 sections showed a separation of 18 to 30 μ and an autoradiograph resolution of approximately 70 μ for ^{35}S and 200 μ for ^{32}P . The remaining sections were not studied.

General Distribution of ^{32}P and ^{35}S in Excised Vascular Bundles. The distribution of the accumulated radioactive material in excised celery vascular bundle tissues (fig 32) was the same for phosphate (fig 1) and sulfate (fig 2, 3), and did not vary down the length of the excised bundle, nor from bundle to bundle, nor from 1 experiment to another. The most radioactive parts of the tissue were 2 areas of the young secondary phloem which formed wings at either side of the bundle (fig 4, 5) in the region where an extension of the fascicular cambium into the interfascicular region might take place. Other young secondary phloem tissues were also very radioactive (fig 1, 3). The remaining areas of tissue, primary and old secondary phloem, young secondary xylem, and old secondary xylem, were much less radioactive (fig 5). The least active tissue was the cortical parenchyma which sometimes remained attached to the bundle (fig 3). Counts of silver grain numbers per unit area were made upon the autoradiograph, above the different types of tissue, so as to obtain a semiquantitative measure of the amount of accumulation occurring in the different tissues within a given section. Typical relative values, per unit area of tissue, in TS sections, were: secondary phloem wing, 100; young secondary phloem, 40; old secondary phloem, 12; young secondary xylem, 10; old secondary xylem, 7; cortical parenchyma, 4. Longitudinal sections confirmed these observations.

Detailed Distribution of Accumulated ^{32}P and ^{35}S in Excised Celery Vascular Bundles. The young secondary phloem which had accumulated most actively was composed principally of young sieve tubes, phloem parenchyma cells and very small companion cells that were difficult to recognize. All cells were

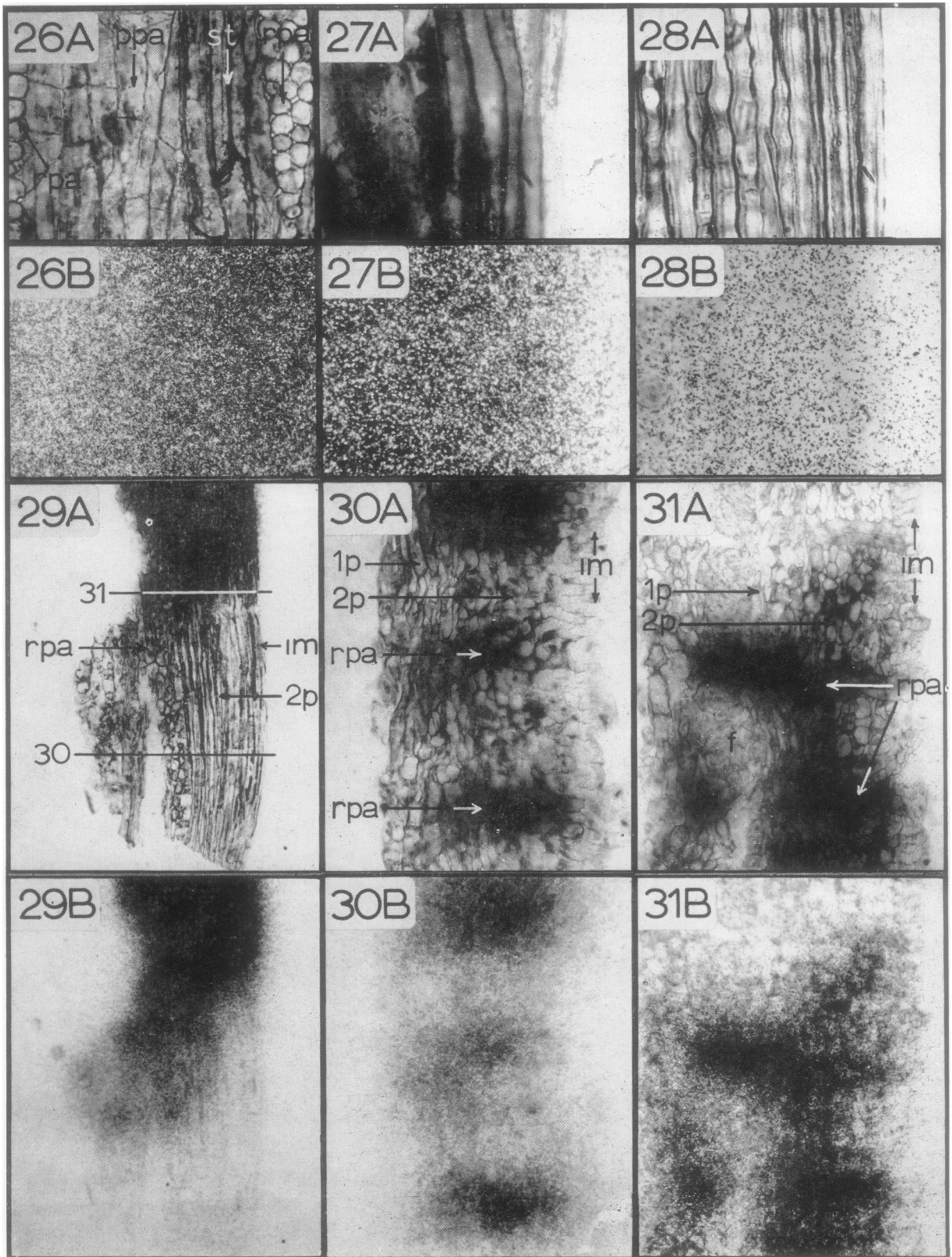
thin walled, and tissue detail was usually poorly preserved. In the older phloem the cell types could be recognized more often. It was then found that the most intense activity was occasionally located in the sieve tubes (fig 8), but that usually, all the cells showed a similar activity (fig 9). The companion cells could not have accumulated at much more than the average rate. Had they done so, they would have constituted very small sources, separated by relatively long distances (30–100 μ); and such a localization of radioactive material should have been apparent, but was not observed. It is also concluded that the sieve tubes and the phloem parenchyma cells had both accumulated actively, and at similar rates. The material accumulated in the xylem was shown to be localized in the xylem parenchyma and not present in the vessels. Since the parenchyma formed only 40 to 50% of the xylem volume, its rate of accumulation must have been twice the average for the xylem, or 5 times that in the cortical parenchyma.

Distribution of ^{32}P and ^{35}S in Translocating Vascular Bundles. When $\text{KH}_2^{32}\text{PO}_4$ or $\text{K}_2^{35}\text{SO}_4$ was supplied to celery leaflets, some was translocated away through the petiole. The petiolar tissue which then contained the most radioactive material was the young secondary phloem (fig 6), particularly the wings (fig 7) of the vascular bundles. Lesser amounts of radioactive material were present in the old secondary phloem, less in the young xylem, and less still in the surrounding cortical parenchyma (fig 6). Thus, the same tissues were involved in translocating in the intact plant as were engaged in accumulating in excised vascular bundles. The presence of radioactive material in the xylem showed that either the xylem cells were contributing to translocation, or that there was a rapid and continuous redistribution of translocated material from the conducting cells of phloem to the adjacent vascular tissues (17).

General Distribution of ^{32}P and ^{35}S in Apple Phloem. The general distribution of the accumulated radioactive material in excised apple phloem (fig 32) was the same for phosphate and sulfate (fig 10, 11). Almost no radioactive material was contained in the occasional clumps of primary phloem fibers (fig 11, 15). Cortical parenchyma cells, where present, showed slight radioactivity. The radioactivities of the primary phloem, old secondary phloem, young sec-



FIG. 19. A, cell and B, silver image showing details of distribution of accumulated sulfate- ^{35}S in all mature cells of excised apple phloem. II, TS, X 145. FIG. 20. Showing a concentration of accumulated sulfate- ^{35}S in the xylem parenchyma cells of the xylem of excised celery vascular bundles. III, TS, X 85. FIG. 21. Showing a general distribution of accumulated sulfate- ^{35}S in all cells, including the sieve tubes, of the secondary phloem and smaller amounts in the ray parenchyma. III, TS, X 75. FIG. 22. A, cell and B, silver image, showing a localization of accumulated sulfate- ^{35}S in sieve tubes of the old secondary phloem of excised apple phloem. I, TS, X 135. FIG. 23. A, cell and B, silver image, showing the distribution of accumulated sulfate- ^{35}S in apple phloem in a region where 4 identifiable sieve tubes lay alongside one another. II, TLS, X 190. FIG. 24. A, cell and B, silver image, showing the distribution of accumulated sulfate- ^{35}S in apple phloem in a region where 4 to 6 identifiable sieve tubes lay alongside one another. II, TLS, X 190. FIG. 25. A, cell and B, silver image, showing the distribution of accumulated sulfate- ^{35}S in apple phloem in a region where there were 4 adjacent columns of sieve tubes. II, RLS, X 200.



ondary phloem, immature (cambial) tissue, and ray parenchyma were much higher but varied slightly relative to one another. Most variations were encompassed within the length of 1 piece of excised phloem tissue: different pieces of tissue from different plant or different experiments showed a generally similar behavior. Occasionally, primary phloem accumulated most rapidly (fig 12); but more often, old secondary phloem (fig 13) or young secondary phloem (fig 14) was the most actively accumulating tissue. Sometimes the cambial tissue accumulated actively (fig 14), but as a rule accumulation there and in the ray parenchyma was less than in the surrounding tissue (fig 12, 13, 19; and fig 12, 13, 21, 22). This variation in behavior of the apple phloem tissues appeared in some cases (fig 12) to be due to physiologically distinct behavior of a continuation of a leaf trace present as a part of the stem phloem layer. Counts of silver grains per unit area were made on autoradiographs over the various tissues of the phloem, to determine the relative accumulation rates of those tissues. Typical relative values, per unit area of tissue in TS, were: old secondary phloem between rays, 45 to 100; young secondary phloem between rays, 30 to 100; primary phloem between rays, 30 to 100; parenchyma rays, 20 to 70; cambial region, 20 to 50; cortical parenchyma, 8; phloem fibers, 2.

It could be shown that this pattern was due to accumulation of the radioactive solutes by the cells, and not to any nonspecific adsorption of ions onto the cell surfaces. The original pieces of living tissue were washed exhaustively and considerable radioactivity remained in them. On the other hand, when dead sections were washed for only 30 seconds, about 95% of the radioactivity was removed, showing that the retention of the radiotracer was a function of the living tissue (fig 27, 28).

Detailed Distribution of Accumulated ^{32}P and ^{35}S in Apple Phloem. The behavior of the individual cell types was studied. Resolution of the ^{35}S autoradiograph, 5 to 15 μ (mounting methods I and III) or 25 to 40 μ (method II), was of the same order as the diameter in transverse section (25–35 μ) of a single cell of most types. With the primary phloem fibers (fig 11, 15), the cortical parenchyma cells and the ray parenchyma cells (fig 18, 21, 22), cells were present as homogeneous, many-celled groups up to 15

cells (300 μ) across, and it can be presumed that the behavior of each cell was like that of the group as a whole. The remaining cell types were present as a mixed population in large pockets bounded radially by the phloem parenchyma rays, and tangentially by the cambial cells and by the primary phloem fibers or the cortical parenchyma. These cells included the sieve tubes, companion cells, and phloem parenchyma cells in their various forms [crystal-containing cells, tannin-containing cells, secondary phloem fiber initials, (7)], and were generally the most radioactive cells in the whole section (fig 12, 13, 21). Since the resolution of the autoradiograph was limiting, and since the cell types were mixed together and sometimes difficult to recognize one from the other, considerable searching and study of several hundred slides was needed before the following conclusions could be drawn as to the part contributed by each of the individual cell types towards the active accumulation of phosphate and sulfate by this group of cells as a whole.

1) Companion cells have dense cytoplasm and many mitochondria (5, 18), and might be expected to be highly active in accumulation. However, accumulation was not carried out principally into the companion cells. These cells occupied only 4% of the tissue volume. They were small (8–12 μ diameter) and separated from one another by relatively large distances (40–120 μ). Any localization of radioactivity in such small, discrete areas would have been strikingly visible in TS autoradiographs (e.g. fig 19, 21). On the other hand, with the resolution obtained, the companion cells could have contained from zero to 2 times the average cell concentration of radioactive material and still have contributed to the observed pattern.

2) Accumulation was not carried out solely or completely by the sieve tubes. As with the companion cells, sieve tubes were scattered enough for some localization of radioactive materials to have been visible in TS autoradiographs had such been the case.

3) Most or all of the phloem parenchyma cells, in all their types, and including immature secondary phloem fibers, must have accumulated at approximately the average rate for the whole mixed group of cells. In many LS sections, it was possible to find up to 6 rows of identifiable parenchyma cells

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FIG. 26. A, cell and B, silver image, showing that sulfate- ^{35}S was accumulated less readily in a region of apple phloem tissue where only parenchyma cells were present than in a region where only sieve tubes were present. II, TLS, X 80. FIG. 27. A, cell and B, silver image. FIG. 28. A, cell and B, silver image, showing the contrasting amounts and patterns of sulfate- ^{35}S activity in duplicate sections from accumulating apple phloem, before (fig 27) and after (fig 28) washing the sections in water for 30 seconds. II, RLS, X 165. FIG. 29. A, cell and B, silver image, showing the absence of accumulated sulfate- ^{35}S in the terminal cells of the excised piece of apple phloem tissue. II, RLS, X 35. Lines mark approximate regions represented in TS by figure 30 and figure 31. FIG. 30. A, cell and B, silver image, showing the absence of accumulated sulfate- ^{35}S from all except the ray parenchyma cells; in transverse sections taken 150 μ from the cut end of the excised apple phloem. II, TS, X 65. FIG. 31. A, cell and B, silver image, showing presence of accumulated sulfate- ^{35}S in all except the young secondary phloem cells, in transverse sections taken 500 μ from the cut end of the excised apple phloem. II, TS, X 60.

adjacent to one another, forming a group about 120 μ across and 150 to 200 μ long (fig 26). The intensity of the autoradiograph image was as high over the center of the group as in the margins (fig 26B). In high resolution autoradiographs (method I, fig 22 and method III, fig 21) recognizable phloem parenchyma cells were associated with an autoradiograph image of average intensity. There was again no indication that any one of the various parenchyma cell types ever contained considerably more or considerably less radioactive material than another.

4) Where areas of sieve tubes lay alongside areas of parenchyma cells, it was found that the sieve tube area had accumulated up to 3 times as actively as had the adjacent parenchyma areas (fig 26). Occasionally, in high resolution autoradiographs of transverse sections, silver grains were seen to be located specifically over sieve tubes (fig 22). In a few longitudinal sections, it was possible to find regions where 4 to 6 clearly identifiable sieve tubes lay adjacent to one another, forming a group approximately 140 μ across by 240 μ long (fig 23, 24, 25). The autoradiograph image tended to be more intense at the center of such a group than at the margins (fig 23). Had the sieve tubes not accumulated any radioactive material, the autoradiograph image should have been almost completely absent at the center of such a group. It was far harder positively to identify sieve tubes in sections mounted by method I. When identification was possible it was found that the sieve tubes were occasionally several times as radioactive as the surrounding parenchyma cells (fig 18, 22).

Thus the estimated relative accumulatory activities of the various cells were: sieve tubes, 100; undifferentiated phloem parenchyma cells, crystal-containing cells, tannin-containing cells and secondary phloem fiber initials, 60; ray parenchyma cells and cambial cells, 35; companion cells, less than 100; cortical parenchyma cells, 6; primary phloem fibers, 1.

Only a little information could be obtained on the distribution of the isotope within the sieve tube itself, although structural details of the sieve tube could be clearly seen in sections mounted by method II (fig 23, 24, 25). Quite often, fibrillar structures were seen (cf 3, 8, 14, 15), sometimes through the length of the cell (fig 16) or sometimes clustered near the sieve plate (fig 17). In high resolution autoradiographs, accumulated sulfate- ^{35}S was occasionally found concentrated in the region of the sieve plate (fig 18). These results suggest that even during careful freeze-drying, the contents of a sieve tube can move towards the sieve plate, possibly through the contraction during drying of cytoplasmic threads attached to the sieve plate (8, 14). A concentrating in the region of the sieve plate of translocated carbohydrate (12) may have arisen in the same way.

Effect of Cell Damage on ^{32}P and ^{35}S Accumulation. When a piece of phloem tissue was excised from the plant, cells along the margins were cut or broken. The effect of this damage on accumulation of ^{32}P and ^{35}S was investigated. TS autoradi-

graphs gave information on the accumulation into cells of the radial and tangential margins, by showing that the regions where ^{32}P and ^{35}S were not accumulated were narrow, about 15 μ or 1 cell wide (fig 13, 27). RLS autoradiographs gave information on accumulation into the cells of the transversely cut ends of the tissue pieces, by showing a wide region at the end where neither ^{32}P nor ^{35}S was present (fig 29). Above the ray parenchyma cells the region was 60 to 200 μ or 3 to 10 cells wide; above the mixed population of sieve tubes, companion cells and phloem parenchyma cells the region was 300 to 1000 μ wide, that is, 1 to 3 sieve tubes, 2 to 8 companion cells and 3 to 12 phloem parenchyma cells wide. Corresponding results were obtained when serial TS sections, taken from the extreme ends of the phloem pieces, were studied. Autoradiographs of sections taken 100 to 200 μ from the end showed small localized areas of radioactivity confined to the ray parenchyma cells (fig 30). Sections taken 250 to 400 μ from the end showed additional radioactive areas covering regions of 20 to 60 adjacent cells in the primary or old secondary phloem (fig 31); and sections taken 600 μ from the end showed radioactivity over all except occasional regions of the young secondary phloem.

These results are interpreted as follows. When the phloem was excised, cut and damaged cells were

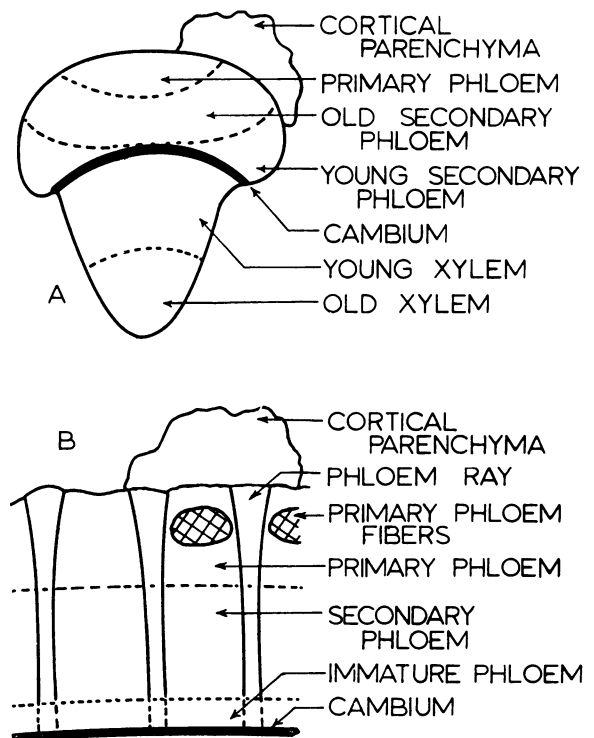


FIG. 32. Diagram of general structure in transverse section of excised celery vascular bundle and of excised apple phloem.

produced; and these were unable to accumulate the radioactive anions. Along the radial and tangential margins of the piece of tissue, only the cut cells themselves were affected in this way; adjacent cells were not damaged, and accumulated normally. However, transverse cuts across the phloem produced a more extensive region in which accumulation did not occur. The extent of the region was partly due to the lengths of the cells (sieve tubes, approximately 350 μ ; phloem parenchyma cells 60–125 μ) but even so, the layer of damage was more than 1 cell deep. Even the essentially isodiametric ray parenchyma cells showed this susceptibility to transverse cuts. The cells appear therefore to have anisotropic properties, suggesting a longitudinal specialization in their function. It was also significant that most of the cells did not behave as individuals in their reaction to damage. Groups of cells accumulated as a unit (fig 31); no region was seen where the phloem parenchyma cells, 80 μ long, had accumulated while the sieve tubes, 350 μ long, had not. Also, sections were seen where all the cells of an older mixed group had accumulated, when in a nearby younger group, none of the cells had accumulated. The ray parenchyma cells clearly behaved as a physiologically distinct as well as a morphologically distinct unit (fig 30). The evidence suggests that the cells of the phloem may tend to function in relatively large groups as physiological units, rather than as a mixture of independent cells of various behaviors.

Conclusions

The accumulated radioactive materials were water-soluble, and it was therefore difficult to meet the 2 requirements of this study: preservation of enough cell detail for cell types to be readily recognized, and a sufficient resolution in autoradiographs for determining the radioactivities of individual cells. Though none of the methods tested was fully satisfactory, their joint use yielded most of the desired information.

It was originally shown (2) that phloem tissues accumulated phosphate and sulfate about 10 times faster than did cortical parenchyma tissue. The present study puts the distinction on a cellular basis, by showing that cells, particularly sieve tubes, of the older secondary phloem accumulated about 20 times faster than the cortical parenchyma cells. It was found that all of the living cells of the phloem were similar in their high accumulatory activity, despite their morphological variety. The behavior of a cell was related to its position rather than to its morphological type. Phloem and phloem ray parenchyma cells, accumulated phosphate and sulfate 10 times faster than cortical parenchyma cells, though the 3 types of cells are similar in appearance. Xylem parenchyma cells are thick-walled and apparently have a structural role to play, yet they accumulated phosphate and sulfate 5 times faster than did adjacent

cortical parenchyma cells, and showed a similar activity in the intact plant.

All of the cells showed differences in accumulatory activity related to the location of the cells in the tissue. The age of the cell appeared to be the most important single factor. For example, the sieve tubes which were most active were usually the young but fully differentiated sieve tubes of the secondary phloem. In the intact celery plant, the same sieve tubes were the most active ones in translocation. Such physiologically mature sieve tubes did not show marked callose development or nacreous wall thickening characteristic of morphologically fully developed sieve tubes (7).

The sieve tubes were among the most actively accumulating cells; and this has significant implications. Since they were capable of any accumulation at all, the sieve tubes must have contained in their volume a region completely bounded by a functional semi-permeable membrane possessing an accumulation mechanism. There is no direct evidence as to whether this region includes the whole cell contents, or is merely confined to a peripheral layer. Indirect evidence, including recent structural studies (3, 5, 9), suggests that the whole cell is involved. The intensity of the accumulation process implies that the sieve tubes were capable of considerable metabolic activity. Such results are not compatible with the sieve tube being a simple conduit. They are in accord with recent structural evidence (3, 9), and with at least 1 recent interpretation of sieve tube structure and function. From studies on living cells, Thaine (16) interprets sieve tubes as having a large vacuolar (fluid filled lumen) region surrounded by parietal cytoplasm and traversed by transcellular strands, and bounded everywhere by a semi-permeable membrane; he regards the observed active protoplasmic streaming as implying high metabolic activity. The source of the metabolic energy could lie outside the sieve tube. Electron microscopic studies show that mitochondria are sparse in the sieve tube and abundant in the companion cell (5) but that there are specialized plasmodesmatal connections between the 2 (3, 18). I envisage the possibility that the companion cell and the sieve tube form a single functional unit, akin to a single unspecialized cell, in which the companion cell represents the cytoplasmic, energy-producing phase, and the sieve tube represents the energy-utilizing, perhaps vacuolar phase, adapted for translocation. The surrounding parenchyma cells are probably directly involved in the translocatory mechanism too: they are highly active, and in excised sections, their accumulatory behavior appears to be linked to that of the sieve tubes.

The view presented here is that specific salt and sugar accumulation mechanisms are directly involved in the phenomenon of phloem translocation; and that many of the characteristic features of the translocation process are due to them. They have been extensively studied, and several of their features are firmly established. 1) They are selective, distin-

guishing between closely similar molecules [e.g. glucose and fructose (1)] or ions [e.g. K^+ , Rb^+ and Na^+ (6)]. 2) They move materials in space (from one side of a semi-permeable membrane to another) and against concentration gradients. 3) They require a supply of metabolic energy in order to function; low temperatures, anaerobiosis and respiratory inhibitors all restrict or prevent accumulation. 4) They can process large amounts of a solute (e.g. up to 100 mg sucrose/g fresh weight phloem tissue/day) and quite large molecules (e.g. sucrose) (2).

These features of accumulation processes include some of the most fundamental features of translocation processes. The main differences are those of magnitude; larger amounts of materials are moved during translocation, and over incomparably longer distances. Still, biologically important compounds are known to pass from 1 parenchyma cell to another, and it is highly probable that they do so through the action of the accumulation mechanisms, from the vacuole of 1 cell, via the cytoplasm to other cells, and ultimately to a vacuole again. There seems to be no fundamental reason why phloem translocation cannot involve a similar process. Sieve tubes actively accumulate solutes. Accumulation may occur into a sieve tube vacuole, though on the other hand, a vacuole as distinct from the cytoplasm may not exist in the sieve tube (3,9), and the entire cell volume may be contained inside a single membrane, the plasmalemma. If Thaine's (15,16) interpretation is correct, a more critical step could be loading of the mobile cytoplasmic fibrils (or microvacuoles contained in them) and a subsequent unloading at a destination.

The main stumbling block in all such theories is to explain how such large quantities of material can be shifted. Most calculations have been made on the assumption that the sieve tubes alone are responsible for all translocation. The results presented here show that all cells of the phloem are similar in their accumulatory and presumably their metabolic activity. Groups of morphologically distinct cells appear to function together as a physiological unit. There was evidence that even phloem parenchyma cells which were isodiametric possessed a longitudinal orientation of function. Young, living phloem fibers appear to have a cytoplasmic organization (13) very similar to that described for living sieve tubes (8,15). All these points taken together support an alternative view that sieve tubes may not be unique in their ability to translocate, and that other cell types and perhaps other tissues may contribute significantly to the movement of metabolites in plants.

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