SIEVE-TUBE STRUCTURE AND TRANSLOCATION IN THE POTATO

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(WITH PLATES II–VII AND ONE FIGURE)

Introduction

The movement of carbohydrates into the potato tuber lends itself to quantitative study. The products of assimilation in the leaves move through the stems and stolon and are condensed to starch within storage cells in the tuber. The stolon may be readily sectioned for measurement; and, knowing the transverse area of its phloem and the rate of development of the tuber, one can calculate the rate of translocation during growth. Photosynthesis is lacking in underground structures, and respiration may be estimated with a fair degree of accuracy.

BIRCH-HIRSCHFELD (**3**) used a similar method to measure transportation from the leaf of *Phaseolus multiflorus;* and DIXON (**10**) calculated a rate of 50 cm. per hour for flow of a 10 per cent. sucrose solution through the phloem of a potato stolon during a 100-day growth period. In a previous paper (**4**) the writer arrived at a somewhat lower rate, 21 cm. per hour, for flow through the total phloem area of the potato stolon during the period of most rapid growth. As respiration losses were not accounted for, this value represents merely the lower limit; actual rates are undoubtedly greater. Many translocation rates have been reported in the literature within the last decade; and, although differing individually, they all approximate this order of magnitude.

Ringing experiments, from the early ones of MALPIGHI (**18**), HALES (**12**), and KNIGHT (**17**) to those more recently performed by CURTIS (**6, 7, 8**), MASON and MASKELL (**19, 20**), and SCHUMACHER (**28**), have indicated that organic nutrients synthesized in the leaves move downward through the phloem tissues of the stem. The writer has proposed (**4**) that the osmotic system described by MÜNCH (**22**) may provide the force necessary to cause this downward flow; and he has attempted to describe a mechanism (**4, 5**) which will fit the quantitative data on translocation and at the same time conform to the anatomy of the plant.

Experiments described by SCHUMACHER indicate that the sieve tubes play an important part in the movement of organic materials out of the leaf. As perforation of the sieve plates has probably no essential rôle in longitudinal movement (**5**), we need some other explanation for the relation of the sieve tube to translocation. A previous paper (**5**) cited evi-

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dence that the permeability of the sieve tubes in cucurbits increases with maturity. The present work discusses the detailed anatomy of sieve tubes in the potato in relation to translocation, and provides data on rates of flow by which the general theory may be further tested.

Phloem anatomy

Although ARTSCHWAGER (**1, 2**) has studied the gross anatomy of the potato, the details of sieve-tube structure have not been described. Because a knowledge of the developmental phases of sieve-tube anatomy must underlie the interpretation of their physiology in relation to translocation, several of these features are included in the present study.

The general vascular structure of the potato resembles that of many herbaceous plants. The bundles are bicollateral. In the stem they tend to remain discrete, being connected laterally by interfascicular cambium; but they fuse more or less in the root and stolon, forming a cylinder of irregular thickness. The phloem is confined to the vascular bundles more strictly than in the cucurbits, but the internal strands follow devious paths and form an anastomosing network in the central region of the stem (**1,** pl. 34–35). Although anastomosis of the phloem strands is also found in the external phloem, sieve tubes do not occur outside the endodermis or starch sheath; nor do the structural differences noted in the cucurbits (**5**) appear.

Sieve tubes are perceptible in very early stages in sections of young stolons cut from imbedded material. At the time the sieve-tube mother cell divides to form sieve tube and companion cell, or soon afterwards, the slime body appears in the cytoplasm. ARTSCHWAGER has described and illustrated these bodies (**2,** pl. 1*D,* 6, 7), showing one of them in a sieve-tube element, the nucleus of which was still in the metaphase (**2,** pl. 6*B*). In the potato these structures differ considerably during their early stages from the slime drops of the cucurbits, only one appearing in each sieve-tube element. In their later development, however, they pass through similar stages, disintegrating as the sieve tube matures.

Plate II, figure 1, shows two of these structures in two successive sieve-tube elements of the young potato stolon. The nuclei are still present, protoplasmic strands have not appeared in the sieve plate, and plasmolysis is apparent in the upper element. Figure 2 shows a slime body in a short connecting element and figure 3 illustrates a later stage. While the slime body and nucleus are present the protoplast seems normal, accumulating neutral red within the vacuole and becoming plasmolyzed in hypertonic solutions. As the element approaches its mature size, however, the nucleus enlarges and disintegrates (fig. 4); the slime body gradually expands, becomes fibrous in structure (plate III, figs. 5, 6), and slowly spreads out

into a tangled mass of gelatinous strands (figs. 7, 8, 9). At this stage, streaming slows down to a plastic flow, and the internal protoplasmic meshwork described in cucurbits (5) is formed (figs. 9, 10). The tangled mass of disintegrating slime may become attached to these moving protoplasmic strands and presents a peculiar writhing motion.

Small leucoplasts imbedded in the protoplasm, and up to this time practically indistinguishable, begin to accumulate condensed carbohydrate, forming spheres (plate III, fig. 9, plate IV, fig. 16, and plate V, fig. 19) which stain pink or violet with iodine. During the early stages of sieve-tube development, while neutral red is readily accumulated, the vacuoles of these cells are distinct and easily distinguished from the parietal cytoplasmic layer. As the nucleus and slime body disintegrate and the leucoplasts enlarge, the phase boundary between vacuole and cytoplasm apparently breaks down; the plastids migrate from the periphery to the central region of the cell, becoming violently agitated by Brownian movement; and neutral red fails to accumulate within the cell. Slime plug formation, which up to this time has not been noted, is readily induced by means of alcohol and other killing agents. In young tissues (plate III, figs. 6, 8, 10, and plate IV, figs. 11, 12) slime plugs are made up of conglomerate masses of partially disintegrated slime bodies and nuclei, but as disintegration becomes complete they are formed of the more homogeneous suspension that results (plate IV, figs. 13, 14, 15).

The protoplasmic layer of the sieve tube reacts differently to different reagents. The condition depicted in figure 14 results from the use of a killing agent composed of three parts of absolute alcohol to one part of glacial acetic acid. That in figure 15 was induced by 95 per cent. alcohol, which causes a severe shrinking of the protoplasm. The internal strands are present, however, and may be densely stained by the use of iodine and anilin blue (plate IV, figs. 13, 16, and plate V, fig. 17). In figure 13 the slime, which is small in volume, has formed on the surface of the sieve plate a compact layer through which the protoplasmic strands project.

By rapidly killing thin sections of potato stolon tissue in this alcohol-acetic acid mixture, one can avoid contraction and staining of the protoplasm; while the suspension of colloidal material within the cell is coagulated to a flocculent reticulum distributed uniformly throughout the cell (plate V, figs. 18, 19). The protoplasm of these mature sieve tubes shows peculiar properties in fresh sections. All streaming movements have ceased, neutral red no longer accumulates, the cells cannot be plasmolyzed, and anilin blue penetrates readily, staining the callus of the sieve plates (fig. 20) and fragments of colloidal material within the cell. Plate VI, figures 21 and 22 illustrate this stage. The former shows a longitudinal section of phloem of potato stolon that has been allowed to accumulate neutral

red for some time. As soon as this picture was taken, a hypertonic sucrose solution was applied under the cover glass; and, as the cells containing neutral red became plasmolyzed, the photograph shown in figure 22 was made. While the sieve tube in the center of these figures was unaffected, the companion cells and several phloem parenchyma cells were severely plasmolyzed.

Although the protoplasm seems to have passed into an inert condition, it is not actually dead, for treatment with alcohol will often cause a radical change or entire cessation of Brownian movement of the plastids; and killing with dilute eosin solution by the method of SCHUMACHER (**28**) will cause rapid callusing of the sieve plates and collapse of the elements. Apparently a condition is reached where distinctions between protoplasm and vacuole become less pronounced; the internal strands and parietal layer are simply portions having greater density or viscosity than the intervening regions.

As the sieve tubes become senile, the plates are heavily callused (fig. 20), as are the side wall pits; and in some instances large spherical masses of callus may partially or almost completely fill the cell lumina. Similar callus formations may be induced by the use of dilute eosin solutions (**28**), and after their development the tubes soon collapse and become obliterated.

The sieve plates of potato develop in the same way as those of cucurbits (**5**), the chief differences being that the protoplasmic connections are smaller, the callus cylinders are less prominent, and definitive callus formation is less pronounced. The optical effect resulting from the focusing of light by the callus cylinders is very striking in potato sieve plates. Figures 23 and 24 show pictures of the same sieve plate taken at slightly different levels, a water immersion lens and a 12× ocular being used. Apparently these plates show an interference phenomenon, the light being blotted out in figure 23 and reinforced in figure 24, for these white spots are much brighter than the original unoccupied field of the microscope.

An oil immersion lens reveals structures similar to those found in cucurbits. The crater-like ends of developing callus cylinders appear in plate VII, figure 25. When stained heavily with anilin blue after being mordanted in iodine, the protoplasmic strands appear as small black dots surrounded by blue callus cylinders (**15**), which in turn lie in an almost unstained cellulose matrix. The differentiation is difficult to photograph because the differences in color are greater than those in intensity and because one can seldom find plates which are clear of adhering protoplasm and flat enough to show more than a few strands in focus at one time. Figure 26 shows a cup-shaped sieve plate with the edge in focus, giving a longitudinal view of the protoplasmic strands; and figure 27 shows them in transverse view. Although the photographs are not very satisfactory, the

actual preparations are quite convincing and confirm the interpretation that these minute dark-staining cores are the actual protoplasmic strands. As they are only 0.3–0.5 μ in diameter, pores within these strands, if such were present, could probably not be detected by means of the microscope.

The slime bodies in potato sieve tubes have only a short existence. During the major portion of their functioning period, the sieve tubes are devoid of these bodies and of nuclei; the limits of the central vacuoles cannot be detected in fresh sections; a great number of starch-containing plastids are suspended within the confines of the parietal layer; and the protoplasm, by its staining and plasmolytic properties, seems to have lost almost completely its property of semipermeability. The companion cells have nuclei and are rather densely filled with protoplasm, while the phloem parenchyma cells are nucleate and contain starch in increasing quantities as they mature.

Translocation studies

As detailed anatomical study seems to render untenable the classical theory of mass flow through open pores in the sieve tubes, we must search critically for another mechanism to explain translocation. As in previous work (**4, 5**), dissection studies have been made on the stems and stolons of the potato.

Phloem exudation is not so pronounced as in the cucurbits, for several reasons. As soon as an incision is made into the phloem, the intercellular spaces of the adjacent parenchyma tissues become filled with sap, acquiring a water-soaked appearance. If another cut is then made near the first, sap will be seen to flow out and wet the surface of the stem with a very thin film that spreads rapidly. These two observations show that the exuded sap has a very low surface tension, differing from that of cucurbits.

If more cuts are made in quick succession near the first one, sap will be seen to accumulate rapidly and run from the wound. If then a cut is made through into the xylem, the sap will be drawn in rapidly and the wounded area will become dry. This shows, as do other experiments, that the phloem exudate for the potato does not coagulate in the same way as cucurbit sap. In addition, one should remember that exudation in the cucurbit stem starts at a rate from three to eleven times that of normal flow (**5**), while in the potato the stem is apparently less elastic, so that little more than the normal rate is observed.

From measurements of the tubers and stolons of potatoes grown in the field, more reliable rates of translocation may be calculated. The plants were of the Russet Rural variety and were grown by the Division of Vegetable Crops of the College of Agriculture, Cornell University, Ithaca, New York.

TABLE I

MEASUREMENTS ON TUBERS AND STOLONS OF POTATO

No.	Tuber			Stolon					
	Fresh wt.	Dry wt.	Dry wt. as % of fresh wt.	Length	Area of transverse section of				Cell wall phloem area ratio
					Stolon	External phloem	Internal phloem $cm.^2 \times 10^{-2}$	Total phloem	
	gm.	*gm.*	*per cent.*	*cm.*	*cm.*^{2}	*cm.*^{2}		*cm.*^{2}	*per cent.*
1	353.0	83.6	23.7	2.0	0.0867	1.98	0.31	2.29	29.7
2	163.0	34.5	21.2	1.8	0.0472	0.63	0.30	0.93	30.2
3	181.5	38.7	21.4	2.3	0.0934	1.28	0.46	1.74	32.7
4	139.5	31.8	22.8	1.7	0.0937	0.93	0.62	1.55	30.7
5	87.0	16.4	18.9	1.7	0.0580	0.83	0.41	1.24	30.2
6	237.6	54.4	22.9	1.4	0.1011	1.03	0.57	1.60	28.6
7	164.5	36.0	21.9	0.5	0.0527	0.63	0.25	0.88	32.4
8	117.2	25.8	22.0	1.3	0.0678	0.85	0.36	1.21	30.1
9	74.2	17.3	23.4	0.8	0.0583	0.60	0.35	0.95	37.8
10	164.3	39.0	23.8	0.9	0.0885	1.11	0.52	1.63	40.1
11	136.0	33.4	24.6	2.2	0.0442	0.55	0.32	0.87	34.2
12	126.6	29.0	22.9	0.8	0.0918	1.11	0.69	1.80	35.6
13	108.2	25.4	23.5	0.2	0.1397	0.90	0.69	1.59	34.5
14	100.5	22.5	22.4	1.3	0.0658	0.97	0.32	1.29	30.7
15	477.2	102.5	21.5	0.8	0.3900	4.21	3.17	7.37	31.1
16	101.8	23.2	22.8	0.3	0.0917	1.52	0.67	2.19	32.5
17	112.5	26.7	23.7	1.8	0.0424	0.47	0.30	0.77	36.6
Total	2,844.6	640.2	.....	21.8	1.6130	19.60	10.31	29.91	.....
Average	165.0	37.6	22.5	1.3	0.0950	1.15	0.61	1.76	32.5

The seed pieces were planted on May 17, and the tubers were dug on September 22, 1930. Weights and phloem areas are given in table I.

The measurements of sieve-tube elements of both inner and outer phloem appear in table II, together with sieve-tube area: phloem area ratios. The latter are somewhat higher than the ratio given in a previous calculation (**4**), while the cell walls occupy a somewhat smaller proportion of the total phloem area.

TABLE II

MEASUREMENTS OF SIEVE-TUBE ELEMENTS IN FIVE POTATO STOLONS

STOLON	EXTERNAL PHLOEM		INTERNAL PHLOEM		SIEVE-TUBE AREA / PHLOEM AREA × 100
	NUMBER OF ELEMENTS MEASURED	AVERAGE LENGTH	NUMBER OF ELEMENTS MEASURED	AVERAGE LENGTH	
		mm.		*mm.*	
1	18	0.114	12	0.123	21.3
2	11	0.117	8	0.109	22.4
3	16	0.159	44	0.108	20.1
4	43	0.107	40	0.088	25.6
5	20	0.096	17	0.082	25.2
Average of all	.....	0.106	.....	.....	22.9

Measurements were made on sieve plates from these stolons, the protoplasmic strands counted, and the sieve-plate areas surrounding the strands computed, as shown in table III.

The average sieve-plate area surrounding each strand was 3.04×10^{-8} cm.^{2} The protoplasmic strands were stained and measured in fully hydrated sections; they averaged 0.3 μ in diameter. The area of the transverse section of each strand was 0.0707 μ^2, and the percentage of the sieve-plate area occupied by strands was $0.0707 \div 3.04 \times 100$, or 2.3 per cent.

Data on the rate of growth of Russet Rural potatoes under field conditions at Ithaca were provided by Professor E. V. HARDENBURG of the Vegetable Crops Division. The potatoes were planted on June 10, and separate lots harvested on August 25, September 15, and October 17. Table IV shows that between the first two harvests the tubers gained on an average 173.0–90.3, or 82.7 gm. This gain in 21 days gives a rate of gain of 3.94 gm. fresh weight per day. According to the dry weight composition given in table I, the daily increment of dry weight was 0.89 gm.

These tables provide a basis for comparing, in potato, the different theories of translocation. The total phloem area in the stolons of the seventeen tubers, as shown in table I, averaged 0.0176 cm.^{2} In testing the

TABLE III

AREAS, NUMBER OF STRANDS, AND AREA PER STRAND VALUE FOR SIEVE PLATES OF POTATO

SIEVE PLATE	AREA CM.$^2 \times 10^{-6}$	NUMBER OF STRANDS	AREA ÷ NO. OF STRANDS CM.$^2 \times 10^{-3}$
1	3.39	134	2.53
2	1.50	68	2.24
3	2.60	101	2.58
4	2.87	105	2.73
5	2.75	111	2.48
6	6.34	201	3.15
7	4.47	158	2.83
8	2.79	58	4.81
9	2.04	34	6.00
10	2.99	56	5.34
11	3.30	67	4.93
12	3.04	103	2.34
13	3.15	69	4.57
14	3.35	63	5.32
15	2.38	46	5.17
16	1.61	61	2.64
17	1.46	67	2.18
18	2.27	99	2.29
19	1.25	53	2.36
20	4.80	121	3.96
21	4.05	108	3.75
22	5.62	151	3.72
23	3.67	130	2.82
24	3.84	133	2.88
25	1.92	110	1.75
Total	77.45	2507	.....
Average	3.10	100.3	3.04

protoplasmic streaming hypothesis, it seems best to calculate the movement on a dry weight basis. The daily increment of 0.89 gm. of dry matter would occupy approximately 0.60 cc. and would have to move in the pure state at a linear rate of $\frac{0.60}{0.0176 \times 24}$, or 1.42 cm. per hour, through the total phloem area. Only about 10 per cent. of the phloem, however, is occupied by streaming protoplasm, one-half of which is flowing in the proper direction; so that if it were carrying with it organic matter equal to its own volume, and were loading and unloading it with machine-like precision, the rate of movement would still have to be 56.8 cm. per hour in order to deliver the required material. But the maximum rate of streaming observed in phloem parenchyma of the potato was 1.8 cm. per hour, while in mature

TABLE IV

GROWTH OF POTATO TUBERS

ROW	PLANTED JUNE 10	HARVESTED	TUBERS PER ROW	YIELD PER ROW	WEIGHT PER TUBER
				gm.	*gm.*
2–7–12	"	Aug. 25	143.5	12,970	90.3
3–8–13	"	Sept. 15	141.0	24,390	173.0
1–6–11	"	Oct. 17	157.3	28,900	184.0

sieve tubes no protoplasmic movements occur in sectioned material. Furthermore, since the length of phloem elements is around 0.1 mm., there would be 100 end walls per centimeter, or, in the average conducting system of 30 cm., about 3000 to be crossed by diffusion. If the total gradient in concentration were from 1 molal to 0, then across any one of these membranes the gradient would be $\frac{M}{3000}$, an insignificant value so far as its quantitative aspect is concerned. Streaming through the protoplasmic strands of the sieve plate would be equally inadequate, because dry matter alone would have to move at a rate of $1.42 \div 0.229 \div 0.023$, or 270 cm. per hour, through the pores occupied by these structures. Even if they were distended by internal pressure, as might be conceivable, they would not suffice, for dry matter alone would have to move 6.2 cm. per hour, or over three times the rate of streaming through the total sieve-tube lumen area. Protoplasmic streaming would seem entirely too slow to act as an effective accelerating or transporting mechanism in the movement of organic nutrients through the potato stolon.

As all of the studies on the structure of sieve plates indicate that the protoplasmic strands traversing them are solid, further testing of the mechanism of mass flow through perforations seems useless. The strands themselves in potato are very slender, being less than 1 μ in diameter; and any pores which could possibly traverse them would necessarily be so small that an immense pressure would be needed to cause the required rate of flow.

When one considers movement through the intermolecular spaces in the hydrated cellulose of which the phloem walls are composed, the pressure again is obviously of an impossibly high order.

Assuming that these intermolecular spaces occur between concentric cylinders (5), so that the formula as modified to apply to flow between parallel planes might be used, and, inserting a plausible value for the pressure, the value derived for the minimal distance between the planes is 0.9 μ.

This is from 10 to 100 times too great for pore spaces in a jell. As there is no justification for assuming that the resistance to flow through cell walls is less than that calculated, the original theory (**4**) must be modified.

Evidence presented in the previous section indicates that sieve tubes increase in permeability with age; and, in potato, soon reach a condition in which they fail to accumulate neutral red and cannot be plasmolyzed with hypertonic solutions. Repeated trials with sugar beet and cucurbit phloem have given the same results. These findings are contrary to the generally accepted (**3, 22**) ones of RUHLAND which earlier led the writer to the conclusion that the sieve tubes played no part in the transport of organic nutrients (**4**). Continued study on this point, however, convinces one that RUHLAND was either dealing with very young sieve tubes or observing other phloem elements.

With the sieve-tube protoplasm completely permeable, the cross walls would present the greatest resistance to mass flow. The pressure necessary to cause movement through the lumina would be almost negligible. If the dry weight increment of the tuber entered in the form of a 10 per cent. solution, it would move at a linear rate of $\frac{0.89 \times 9}{0.0176 \times 24} = 19.0$ cm. per hour through a passage equal, in cross-section, to the total phloem. Through the 22.9 per cent. of this area occupied by sieve tubes, it would move at a linear rate of 83.0 cm. per hour, or 0.023 cm. per second, requiring a pressure difference of 0.0035 atmospheres per sq. cm. through the stolon.[2]

A study of the volume relationships of the potato plant indicates that the stolon is a region where the transverse phloem area is constricted. The pressure gradient necessary to cause flow through sieve-tube lumina in the entire plant, therefore, is probably not more than ten times this value, the difference between this and the actual pressure in the phloem being required to overcome the resistance of cross walls.

If the sieve-tube end walls were so arranged as to act as a complete obstruction across the total phloem at frequent intervals, then the area available for longitudinal flow through wall material would be the sum of the sieve-tube lumen area plus the sum of the phloem wall area. An average value for this sum would be, from tables I and II, 55.4 per cent. of the total phloem and the average rate through this area 34.3 cm. per hour. In the potato, however, many of the sieve plates are not transverse and seldom do many of them occur in any one plane. By this arrangement a much

[2] Calculated according to POISEUILLE'S equation, $P = \frac{8 R_l n l}{r^2}$ where P = pressure in dynes per sq. cm.; R_l, linear rate of displacement = 0.023 cm. per sec.; n, viscosity of the solution = 0.012; l, average length of stolon = 1.3 cm.; and r, average sieve-tube radius = 0.0009 cm. $P = \frac{8 \times 0.023 \times 0.012 \times 1.3}{0.81 \times 10^{-6}} = 3543$ dynes or 0.0035 atmospheres.

greater surface is exposed, reducing even more the actual rate of movement through wall material. This is brought out in text figure 1 *A*, showing the strictly transverse arrangement, and *B*, the situation (greatly foreshortened) as it occurs in potato.

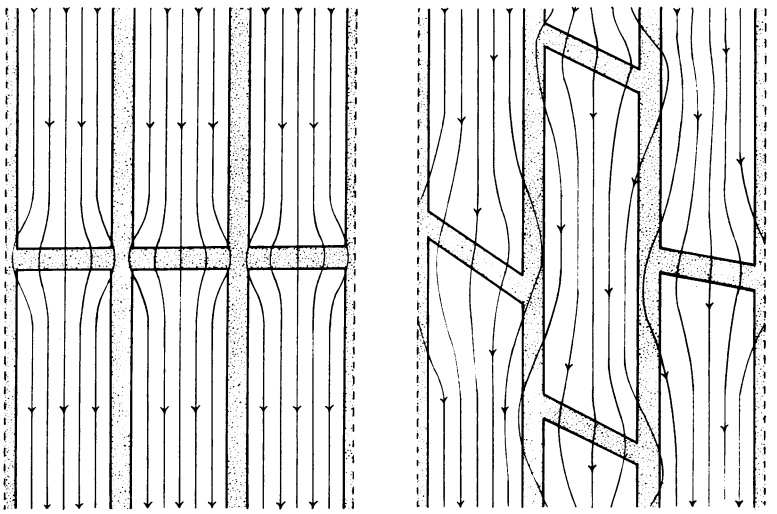

FIG. 1. Diagrammatic representation of flow through potato phloem.

The arrows in these diagrams represent the flow of solution, the distance between them being inversely proportional to the rate of flow. From *B* it can be seen how the actual rate of flow through the wall material may be greatly decreased, reducing the pressure required to maintain movement. By using this reduced rate in the modified formula (5) and substituting the volumes and pressures computed for sap concentrations of 10, 15, and 20 per cent., values have been calculated for the minimal distance between parallel planes which would accommodate this flow. As the stolon is a constricted region, a greater pressure gradient per unit length is required. The values are computed on the assumption that 10, 20, or 50 per cent. of the total available pressure is utilized within this limited region.

These values (table V) show that by assuming ideal conditions, namely, a high concentration in the flowing sap and a high utilization of energy within the limited stolon region, one may arrive at dimensions for capillary spaces which approach the probable values. Respiration has not been considered, and a zero pressure is being assumed at the "sink" or storing parenchyma cell. If, on the other hand, hexose sugars and organic acids of low molecular weight were present in the sap, considerably higher pressures might be expected.

TABLE V

DIMENSIONS OF CAPILLARY SPACES REQUIRED FOR CONDUCTION IN POTATO STOLON; CALCULATIONS BASED ON SUCROSE

SOLUTE	CONC.	OSMOTIC PRESSURE AT 25° C.	DIMENSIONS OF CAPILLARY SPACES DISTANCE BETWEEN PARALLEL PLANES		
			PRESSURE UTILIZED		
			10 PER CENT.	20 PER CENT.	50 PER CENT.
per cent.	*molal*	*atm.*	μ	μ	μ
10	0.33	8.5	0.066	0.046	0.029
15	0.52	13.5	0.043	0.031	0.019
20	0.73	19.4	0.033	0.023	0.015

The potato stolon is apparently a very efficient conducting organ; and if a mechanism can be found which will satisfactorily explain its functioning, other cases should present no difficulty. Longitudinal movement through structures lacking mature sieve tubes seems to take place along the walls, and dissection experiments indicate that in growing shoots and root tips this movement is not limited to the phloem but spreads throughout the structure. Apparently those tissue layers which, in the mature stem, limit flow to the phloem, have not yet become functional, the epidermis alone serving to check loss by leakage.

MÜNCH (**22**) has suggested that lateral movement from mesophyll to vascular tissue and from vascular tissue to storage tissues or secondary meristems takes place from living cell to living cell by way of the plasmodesma. In that case, these connecting strands should be abundant in the parenchyma cells of the potato tuber, especially in the vicinity of the phloem strands. Examination of these tissues showed this hypothesis to be correct; and figures 28, 29, and 30 illustrate these structures, stained with gentian violet and iodine. If the plasmodesma are actually wall structures, as JUNGERS (**16**) has recently suggested, it seems strange that when the wall is swelled by the use of 30 per cent. sulphuric acid to double its original thickness (fig. 31), the plasmodesma should undergo no greater change in length than those treated with only 5 per cent. acid (fig. 32).

Upon entering the tuber, the vascular tissue of the stolon multiplies rapidly in amount and the rate of flow is materially reduced. Lateral diffusion from the strands diminishes this rate even more, longitudinal movement becoming relatively slow.

Measurements were made on two tubers; and the resultant values, given in table VI, aid in the determination of these reduced rates.

TABLE VI

MEASUREMENTS ON POTATO TUBERS

TUBER NO.	WEIGHT OF TUBER	LENGTH OF TUBER	TRANSVERSE DIAMETERS OF TUBER	VOLUME OF TUBER	AREA OF TRANSVERSE SECTION	NUMBER OF VASCULAR BUNDLES	AREA OF VASCULAR BUNDLES	AREA OF VASCULAR BUNDLES AS PER CENT. OF TOTAL
	gm.	*cm.*	*cm.*	*cc.*	*cm.*^{2}		*cm.*^{2}	*per cent.*
1	237.0	9.4	7.2 × 6.1	220.3	33.4	827	2.07	6.2
2	242.0	9.7	7.5 × 5.5	225.0	32.5	806	1.73	5.3
Sum	479.0	19.1	.....	445.3	65.9	1633	3.80	.....
Average	239.5	9.5	7.3 × 5.8	222.6	32.9	816	1.90	5.8

As the area occupied by phloem in the stolon is roughly proportional to the weight, for these tubers it would be about $\frac{239.5}{165} \times 0.0176$, or 0.0255 cm.^{2} Dividing the area occupied by phloem in the vascular bundles of the tuber by this value, $1.9 \times 2/3 \div 0.0255$, we find that the phloem expands approximately 50 times within the tuber at its maximum girth. The mean expansion should be about two-thirds this value; and the mean rate of flow within the tuber, if one considers the continual loss by diffusion, should be around 0.285 cm. per hour. The average length of vascular bundle in these tubers is 5.3 cm., and the average time required by the solution to reach its final location within the phloem is $\frac{5.3}{0.285}$, or 18.6 hours.

In calculating the rate at which solute diffuses from the phloem to its final destination within the storage cells, the mean distance must be found by computing the radius of the circle whose area is one-half that of the total area of parenchyma surrounding each vascular strand. Correcting for the area of the bundle itself and for the increase in total area due to growth, the mean distance is 0.047 cm., and the mean rate of movement 0.0025 cm. per hour.

As protoplasmic streaming is common within the parenchyma cells of growing potato tubers, it might possibly explain the movement of solutes from end to end of these cells, leaving only the thickness of walls to be traversed by diffusion. According to measurements, the walls occupy about 3.7 per cent. of the distance. Then $0.047 \times 0.037 = 0.00174$ cm., the distance to be traveled by diffusion. If streaming accounts for an effective movement of 0.018 cm. per hour, the materials would move 96.3 per cent. of the distance in 2.5 hours; 16.1 hours would remain for diffusion; and $0.00174 \div 16.1 = 0.00011$ cm., or 1.1 μ per hour, the rate of diffusion.

This rate proves to be 3.01×10^{-8} cm. per second, or, through unit cross-sectional area, 3.13×10^{-8} gm. per second. If the concentration decreased 0.1 molal within the 0.047 cm., the diffusion constant would be 5.5×10^{-10} for movement across the total area, or 0.11×10^{-7} for diffusion through the 5 per cent. occupied by plasmodesma. This value is about 100 times smaller than that for diffusion through water, and closely approximates that obtained by STEWARD (**29**).

MÜNCH has calculated rates of movement in leaf tissues. From the data on potato (**22,** p. 87), according to his method, a rate of 6.25 μ per day for movement within the leaf was obtained. As plasmodesma in the leaf are confined to pits, and as acceleration by streaming would be confined to the parietal layer, the actual rate of movement is probably many times this. If it were as great as 100 times this value, it would be only

625 μ per day, or 26 μ per hour. Dividing the mean distance of diffusion, 62.5 μ, by 26, we find that 2.4 hours would be the average time consumed. Streaming, common again in these tissues, would carry materials 95 per cent. of the way in 0.3 hours; and the remaining distance, 3.125 μ, divided by the remaining time, 2.1 hours, gives a diffusion rate through the plasmodesma of 1.5 μ per hour.

As the values obtained by these various calculations all lie within the realm of possibility, apparently this combination of diffusion across walls along plasmodesma, acceleration by streaming within living cells, and mass flow through vascular tissues may satisfactorily explain the conduction of organic nutrients in the potato.

Discussion

The studies made on potato and on cucurbits indicate that translocation by diffusion (**26**), protoplasmic streaming (**6, 7, 8, 9**), young sieve tubes (**24**), conducting parenchyma cells (**11, 26**), or perforations through the sieve plates (**23, 22**) can play little part in phloem exudation or in the rapid transport of carbohydrate into the tuber. While the mechanisms of the first two theories seem too slow and the second two are incompatible with present concepts of the living protoplasmic structures of cells, the last, in spite of its classical position, fails because critical study has not demonstrated the presence of open pores in sieve plates.

The errors of the early workers are not difficult to understand in view of the instruments they used. To HARTIG (**13**), NÄGELI (**23**), VON MOHL (**21**), and SACHS (**26**), the sieve plates undoubtedly appeared to be truly perforated, and the phenomenon of phloem exudation seemed to confirm this conclusion. One wonders, however, why no one before SCHMIDT (**27**) pointed out the fallacy of this view, and why his work has aroused so little comment.

SACHS' concept of conducting parenchyma (**26**), although based almost entirely upon circumstantial evidence and although partially refuted by HEINE (**14**), is still accepted by HABERLANDT (**11**) and others.

PRIESTLEY'S idea of movement by streaming through differentiating sieve tubes (**24**) seems difficult to accept because, in many leaves having practically no secondary tissue, translocation continues long after differentiation has ceased, and because, in such structures as the potato stolon, movement would be restricted to an insignificant fraction of the phloem.

Although the evidence for increasing permeability of the sieve tube with maturity is only fragmentary at present, if subsequent studies, using various methods and many plants, confirm this observation, it should prove ex-

tremely important in relation to rapid translocation. No other mechanism can so well explain the limiting cases exemplified in this study.

Summary

1. Because the potato is particularly favorable for translocation studies, the anatomy and physiology of phloem tissues in this plant have been investigated.

2. Young sieve tubes of the potato are nucleate and display the characteristics of normal living cells.

3. As the sieve-tube elements mature, the nuclei and slime bodies disintegrate; and the protoplasm apparently changes its organization, becoming more and more permeable.

4. Slime plugs are shown to be artifacts formed by the action of killing agents upon the vacuolar contents; pores within the protoplasmic strands of the sieve plates could not be demonstrated.

5. Phloem exudate from the potato has a low surface tension, does not coagulate rapidly, and seems to emerge at a rate of flow that would account for normal translocation.

6. Measurements indicate that a 10 per cent. sucrose solution would have to flow 19 cm. per hour through a conduit equal in transverse area to the total phloem to provide for tuber formation.

7. The theories of protoplasmic streaming and pressure flow through phloem walls seem inadequate to explain this rate of movement.

8. Sieve-tube lumina apparently afford the most available channels for this movement, the parietal protoplasm offering little resistance. Capillary spaces of from 0.01 to 0.06 μ would allow movement across end walls under the available pressure.

9. The phloem increases greatly in cross-sectional area within the potato tuber. The rate of flow is correspondingly reduced.

10. Protoplasmic streaming may accelerate lateral movement across non-vascular tissues.

11. Calculations indicate a rate of 1.1 μ per hour for diffusion along plasmodesma of cross walls in the tuber. The corresponding diffusion rate in the leaf would be 1.5 μ per hour.

12. Diffusion along plasmodesma of cross walls and acceleration by protoplasmic streaming within non-vascular tissues, combined with pressure flow through permeable sieve tubes and phloem walls within specialized conducting organs, seem most satisfactorily to explain translocation in the potato.

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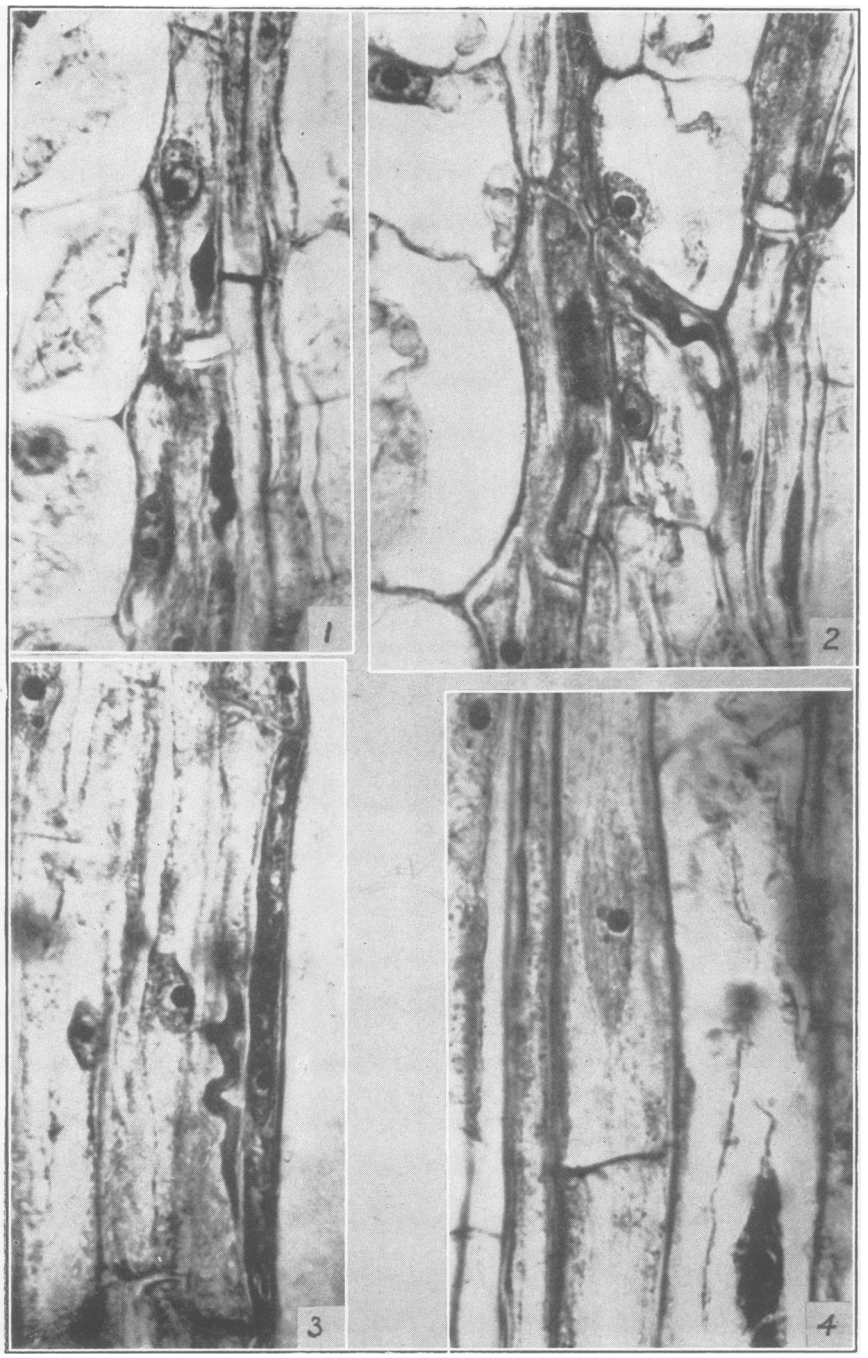

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Explanation of Plates

PLATE II

Fig. 1. Longitudinal section of phloem strand in young potato stolon, showing sieve-tube elements soon after division of sieve-tube mother cell; imbedded material stained with haematoxylin and light green.

Fig. 2. Slime body in short sieve-tube segment of anastomosing internal phloem strands of young potato stolon; same stain as for fig. 1.

Fig. 3. Slime body in developing sieve-tube segment. Note nucleus and densely staining contents of companion cell; same stain as for fig. 1.

Fig. 4. Sieve-tube element in older stolon; nucleus is disintegrating, being enlarged and only lightly stained; staining like fig. 1. All × 750.

PLATE III

FIG. 5. Disintegrating slime body, showing gelatinous strands starting to separate. ×1200.

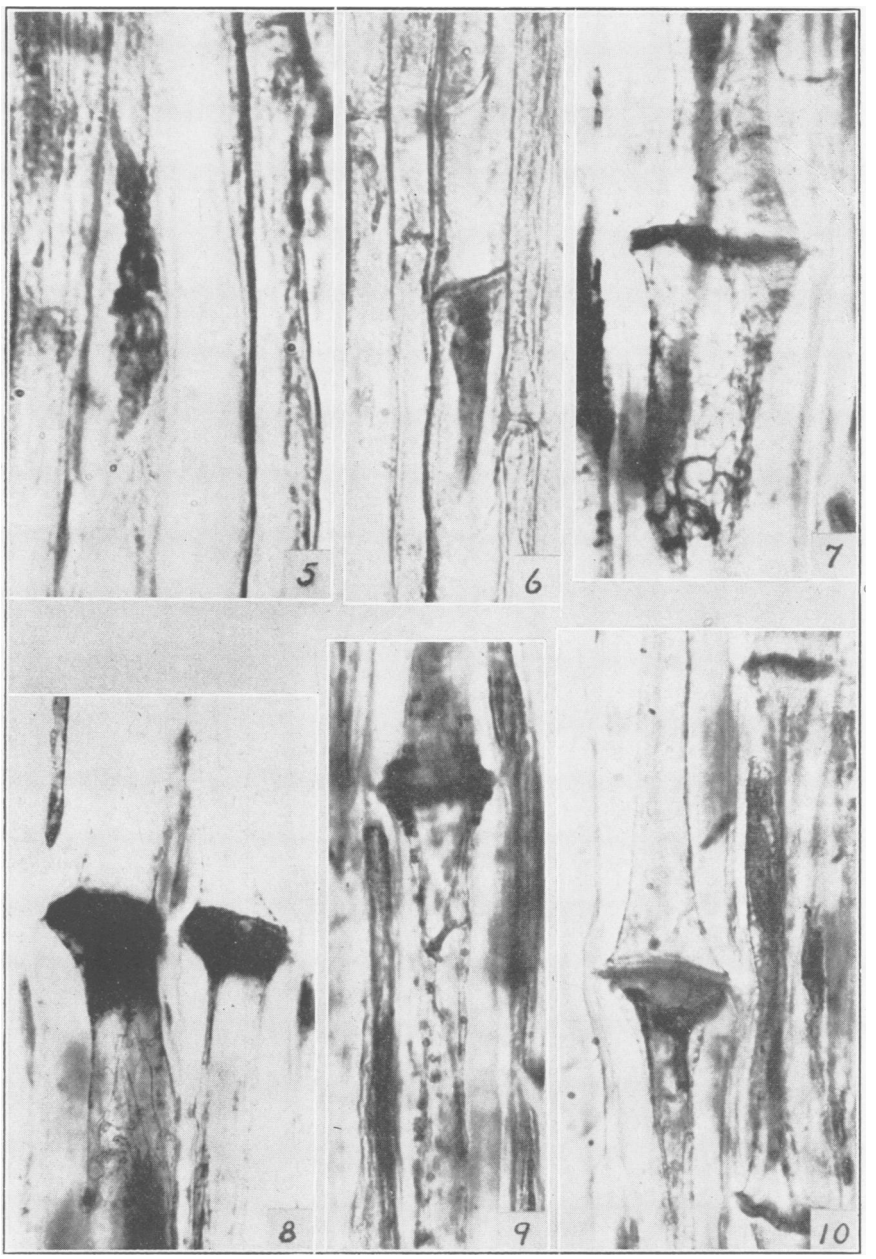
FIG. 6. Disintegrating slime body lodged against sieve plate. Figs. 5 and 6 stained with haematoxylin and light green. ×750.

FIG. 7. Disintegrating slime body, showing tangled mass of gelatinous strands lying within the cell lumen. ×900.

FIG. 8. Slime body further disintegrated. Strands have become threadlike, and much of the albuminous suspension has accumulated at the sieve plate. ×750.

FIG. 9. A few remnants of the slime body may be seen in this well developed sieve tube. Plastids have accumulated considerable carbohydrate material. ×750.

FIG. 10. Remnants of the slime body adhering to the internal protoplasmic structure of fully developed sieve tube. Figs. 7–10 are fresh hand-sections with IKI and anilin blue. ×750.



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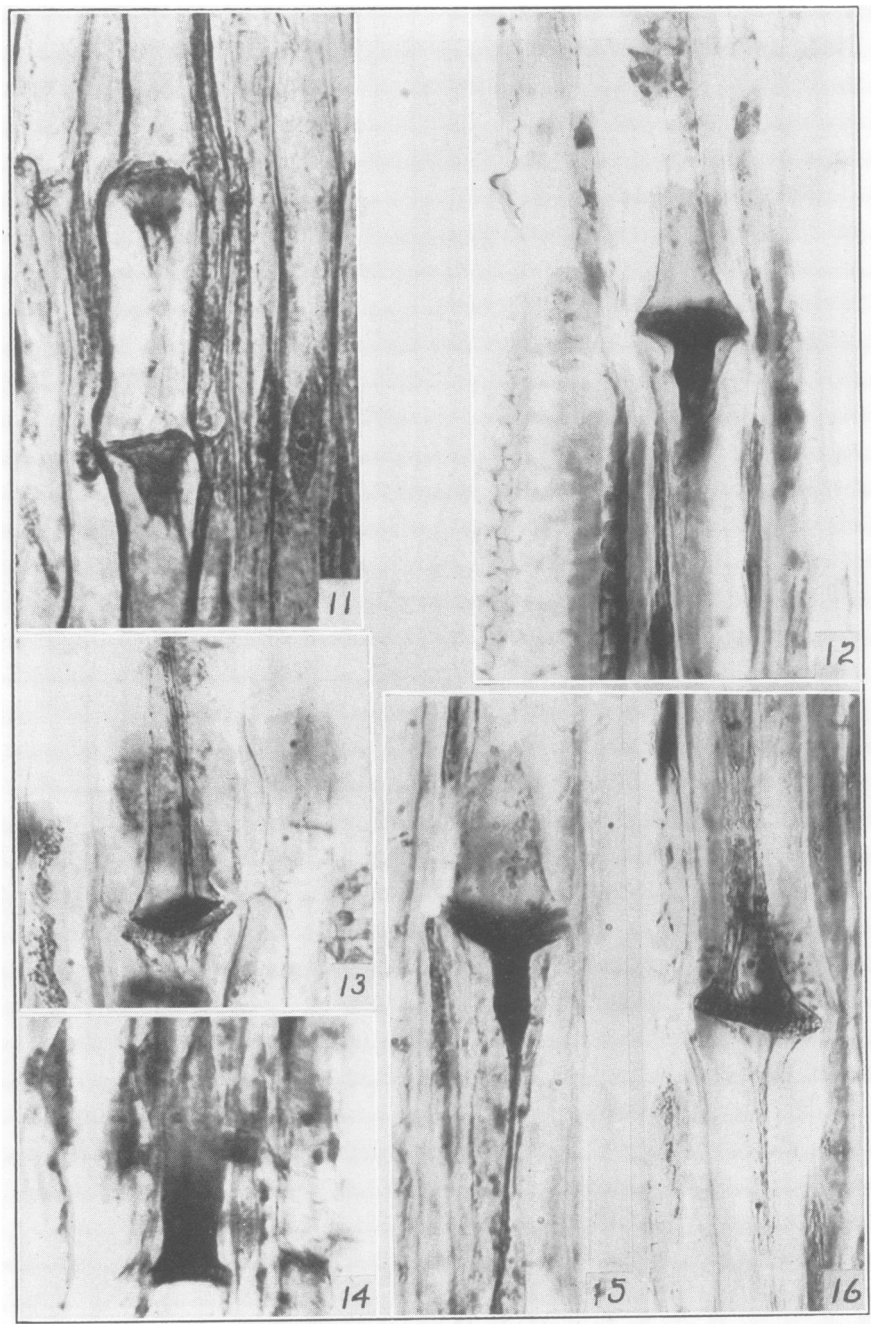

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PLATE IV

FIG. 11. Slime accumulations in young sieve-tube elements of potato stolon. Fragments of slime bodies may be seen within the accumulated masses. Haematoxylin and light green.

FIG. 12. Slime plug in a young, fully differentiated sieve tube. Parietal layer may be seen distinct from the slime.

FIG. 13. Slime accumulated as a thin, compact mass of homogeneous material lodged closely against the sieve plate. Internal protoplasmic strands may be seen projecting through the slime.

FIG. 14. Slime accumulation in a sieve tube killed by applying a mixture of 3 parts of absolute alcohol and 1 part glacial acetic acid to one end of the section. The mass is dense near sieve plate but less and less concentrated toward center of cell. Parietal layer is slightly contracted.

FIG. 15. Dense slime plug in old sieve tube formed by killing with 95 per cent. alcohol. Parietal protoplasm is contracted and surrounds the slime.

FIG. 16. Internal protoplasmic strands in mature sieve tube. Plastids above the plate. Figs. 12–16 stained with anilin blue and iodine. All $\times 750$.

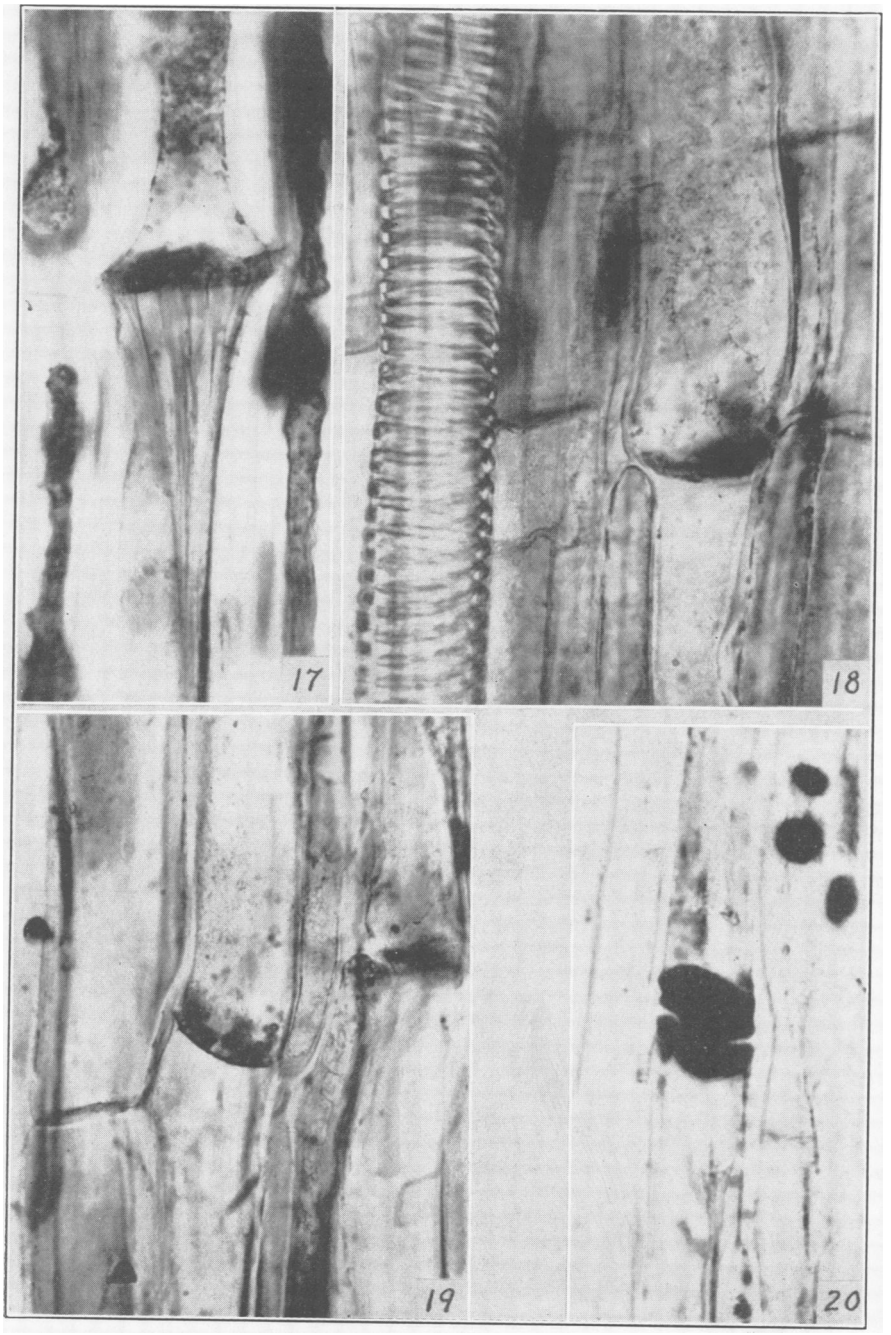
PLATE V

FIG. 17. Internal protoplasmic strands in mature sieve tube. Under the microscope the threads could be followed directly to the strands of sieve plate. Hand section killed and mordanted in IKI and stained with anilin blue. × 900.

FIG. 18. Sieve tube and spiral vessel of potato stolon. Section killed with absolute alcohol and acetic acid. Parietal and internal protoplasm unstained and not contracted. Sieve-tube lumen contents coagulated to a flocculent reticulum which completely fills the cell. Dark mass at the sieve plate is composed of plastids which were out of focus. × 750.

FIG. 19. Another sieve tube in same section as fig. 18, showing plastids more clearly. In living material these plastids are rapidly agitated by Brownian movement. Figs. 18 and 19 stained with anilin blue and iodine. × 750.

FIG. 20. Definitive callus on sieve plate stained with anilin blue. × 750.



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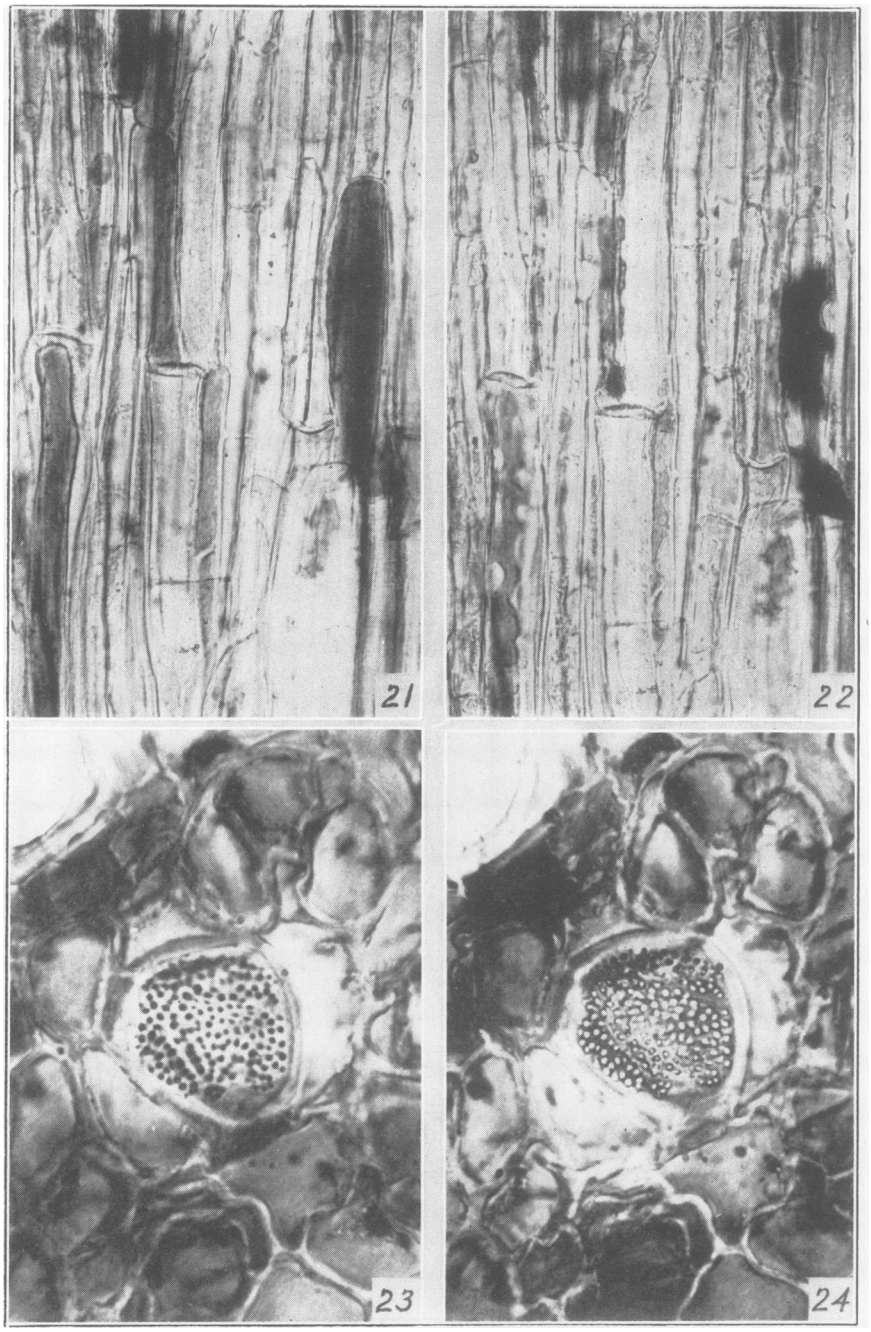

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PLATE VI

FIG. 21. Longitudinal section of potato stolon, showing sieve tube, companion cells, and phloem parenchyma in living condition after having accumulated neutral red for a short period of time. ×325.

FIG. 22. Same section after application of a hypertonic sucrose solution under the cover slip. Sieve tube in center of picture remains unchanged, while companion cells and phloem parenchyma are severely plasmolyzed. ×325.

FIGS. 23, 24. Sieve plate of potato stolon stained with iodine and anilin blue as viewed through a water immersion lens. With the focus high (fig. 23), the protoplasmic strands and surrounding callus cylinders appear as black spots. If the focus is slightly lower, the dark spots give way to small bright spots (fig. 24) which are considerably brighter than the unoccupied field of the microscope. ×900.

PLATE VII

FIG. 25. Young sieve plate, showing crater-like ends of callus cylinders which surround the protoplasmic strands. Stained with iodine and dilute anilin blue. ×800.

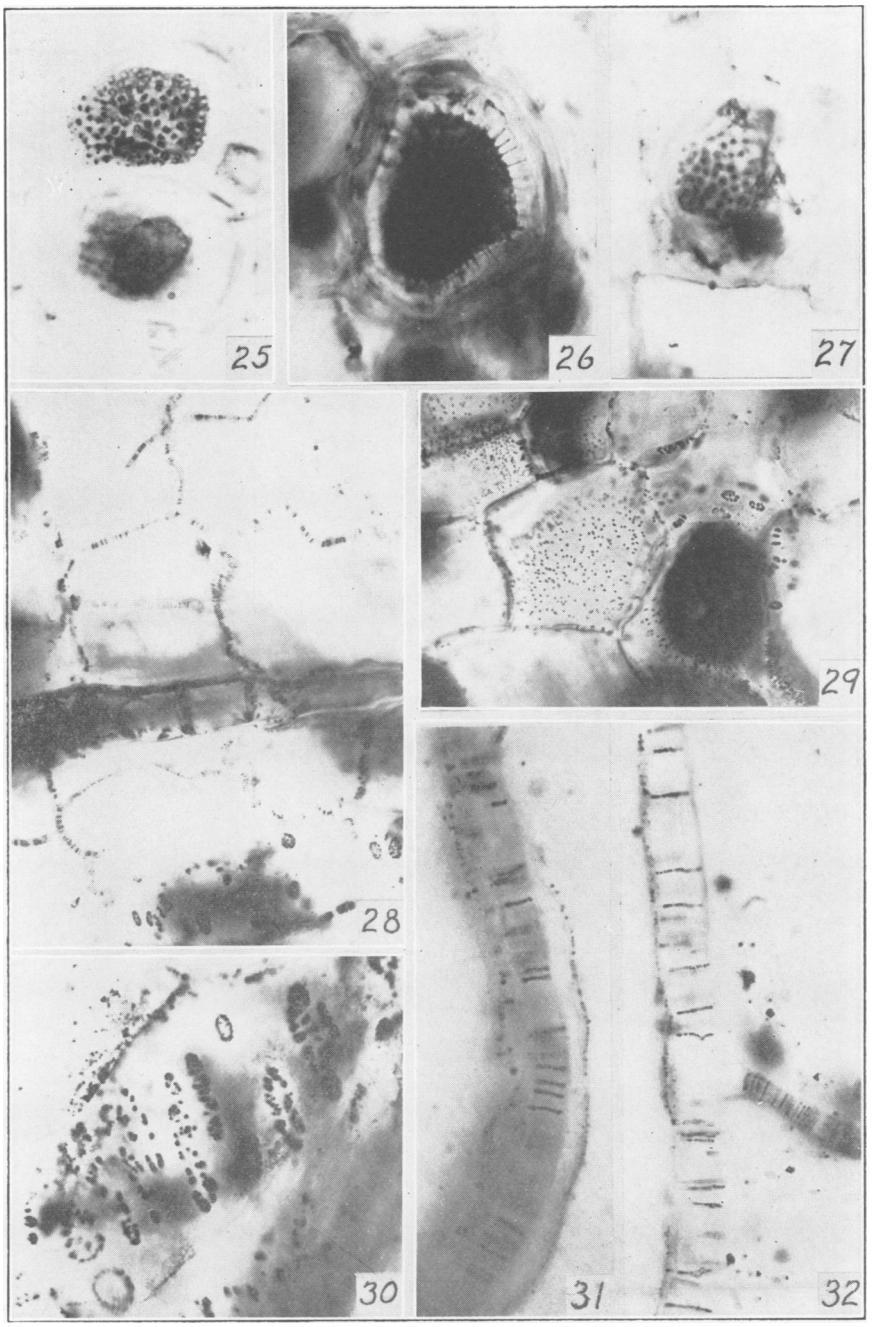
FIG. 26. Cup-shaped sieve plate, showing the narrow protoplasmic strands. Callus cylinders cannot be seen in this photograph because of lack of color differentiation; in the original section they could be seen. ×850.

FIG. 27. Transverse section showing sieve plate. Protoplasmic strands surrounded by callus cylinders. The sections in figs. 26 and 27 were stained with a strong solution of anilin blue after being mordanted in a very dilute iodine solution. Protoplasmic strands show black, and callus cylinders blue. A yellow filter was used in taking this photograph. ×850.

FIGS. 28, 29. Plasmodesma in potato tuber tissue. ×325.

FIG. 30. Plasmodesma of pits, more highly magnified. ×950.

FIGS. 31, 32. Thick walls of pith parenchyma of potato, showing plasmodesma in longitudinal view. Figs. 28–32 mordanted in iodine and stained with gentian violet in 5 per cent. sulphuric acid.



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