STUDIES IN TRANSLOCATION

I. THE RESPIRATION OF THE PHLOEM

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Summary

Comparative studies have been made of the respiratory metabolism of the vascular and ground tissues of the shoots of four species, *Apium graveolens*, *Cucurbita pepo*, *Vitis vinifera*, and *Verbascum virgatum*. As far as examined the respiration of the phloem tissue in each species was qualitatively identical with that of the surrounding ground tissues, and generally similar to that of other plant tissues. There was no evidence of a "peculiar metabolism" in the phloem; but the respiration rate of phloem tissue, per unit fresh weight, as measured by oxygen uptake, is calculated as being from 5 to 40 times higher than that of the ground parenchyma tissue of the same shoot. Since the differences in rates can be largely accounted for by differences in protein nitrogen, it is concluded that the higher respiration rates of the phloem are the result of its having more protoplasm per unit volume of tissue than the parenchyma.

I. INTRODUCTION

In recent years it has been suggested from studies with the light-microscope (Currier, Esau, and Cheadle 1955), and with the electron-microscope (Hepton, Preston, and Ripley 1955; Schumacher and Kollman 1959), that the sieve element on reaching anatomical, and it is assumed, physiological maturity, possesses a protoplast with possibly a peculiar metabolism which may be responsible for phloem transport. The high levels of enzymatic activity associated with the phloem tissue (Kuprevich 1949; Kendall 1955), and the very high respiration rates which have been calculated for the phloem (Kursanov and Turkina 1952; Kursanov, Turkina, and Dubenina 1953) have led the Russian workers to the conclusion that "sucrose or some substance to which it is readily converted in the conducting cells, is transported in the phloem by a process driven by the metabolic activity of the conducting cells" (Kursanov and Turkina 1952).

In view of the scarcity of information concerning phloem respiration, and since our own electron-microscope investigations (Duloy 1960; Duloy, Mercer, and Rathgeber 1961) do not support the conclusions drawn by other workers about the structure of the mature sieve-element protoplast, we have undertaken comparative studies of the respiration of phloem and ground tissues of four species. Most of the work was carried out using the stem of *Cucurbita pepo* (Duchesne), the mammoth cattle pumpkin. Some observations were made on tissues from the stem of *Vitis vinifera* (L.), the petiole of *Apium graveolens* (L.), and the midrib of the leaf of *Verbascum virgatum* (Stokes).

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II. Materials and Methods

(*a*) C. pepo

Seeds were planted every 2 weeks during September–April in open plots, and stems were harvested 40–60 days from planting. The stem has a distinctive anatomy with two concentric cylinders, each of five bundles, the large outer bundles alternating with the small inner bundles. The pith is hollow. The bundles are bicollateral. A layer of cambiform tissue is present on either side of the xylem, but from studies with the light-microscope it was found that cambial activity is confined mainly to the outer side. In the sixth and older internodes a band of sclerenchyma is present in the pericycle. No further cambial activity takes place after the production of the sclerenchyma layer. Bundles in this condition are referred to as "mature". Mature internodes are 15–20 cm in length, and 2–3 cm in diameter.

To harvest, a runner, including some 10 or 12 internodes, was cut from the vine, the cut end being placed in distilled water immediately. From this length of stem the first three mature internodes (sixth, seventh, and eighth) were taken separately and cut into lengths of 1 cm before being split vertically into two. The bundles of the inner ring were removed by two or three vertical cuts, and placed in a covered weighing bottle. The remaining stem tissue was trimmed so as to include only parenchyma, and placed in a second weighing bottle.

(*b*) A. graveolens

Material was obtained fresh from the market as required. This species was chosen because it is possible to isolate collenchyma from the petiole, as well as vascular tissue and parenchyma. The xylem consists mainly of vessels and xylem parenchyma. Abaxial to the phloem is a bundle cap composed of a type of collenchyma, less thickened than that in the cortex.

To prepare, the strips of cortical collenchyma and of vascular tissue were pulled separately away from the petiole and cut into 2-cm lengths. From the remainder of the petiole 2-cm segments of parenchyma were prepared.

(*c*) V. vinifera

Vines were grown in the open and were used for respiration studies between November and April. Canes some 50 cm in length were cut from the vine, the cut end being placed in distilled water. From this length internodes were cut one by one for treatment. The periderm was first peeled away exposing a layer of secondary phloem consisting of two comparatively thick crescents joined on either side by narrower strips. The crescents were cut into 1-cm lengths and slit into two.

(*d*) V. virgatum

The plants were growing in a loose rubble of ash and slag. When required, the plants were uprooted and the root systems were placed in distilled water. The leaves were removed one by one with a razor-blade. The vascular strand in the midrib was isolated by sharply bending the petiole and then pulling the petiole away from the

lamina. Both the xylem and phloem consists of radial rows of conducting cells* separated by bands of much-thickened collenchyma. Collenchyma also forms a bundle cap on either side of the bundle.

For all species, samples of 0·2–0·3 g fresh weight of each tissue were weighed from bulk lots in covered bottles, and either were transferred directly to Warburg vessels, or aerated overnight in water or unbuffered sucrose solutions. The composition of the vascular tissues as dissected are shown in Table 1. These values are used as the basis of calculations of the respiration rates of the phloem.

TABLE 1

PROPORTIONAL AREAS OF THE VARIOUS COMPONENTS OF THE VASCULAR TISSUES OF FOUR SPECIES AS DISSECTED FOR RESPIRATION STUDIES

	Tissue	% of Total Tissue	% of Living Tissue
C. pepo	Lignified xylem*	22·2	0
	"Normal" parenchyma†	44·4	57
	Xylem parenchyma plus cambiform tissue	3·5	4·5
	Phloem	29·9	38·5
A. graveolens	Lignified xylem*	16·5	0
	Xylem parenchyma	16·8	20
	Cap collenchyma	44·3	53·2
	Phloem	22·4	26·8
V. vinifera	Phloem conducting cells‡	32·1	41
	Phloem fibres	21·4	0
	Rays	46·5	59
V. virgatum	Lignified xylem*	26	0
	Phloem conducting cells‡	22	30
	Phloem collenchyma	52	70

* i.e. includes vessels, tracheids, fibre-tracheids, and fibres.

† i.e. that which is identical and continuous with the surrounding ground parenchyma whose respiratory activity was measured.

‡ i.e. sieve tubes, companion cells, phloem parenchyma. Term used to distinguish these cells from phloem fibres or phloem collenchyma.

(e) *Measurement of Respiration*

Respiration rates were determined by measuring oxygen uptake at 25°C using the standard Warburg technique. The direct method of Warburg and Dixon was used to determine respiratory quotient (R.Q.) values. Na_2HPO_4–KH_2PO_4 buffers (M/30), with and without 0·03M sucrose, were used in all experiments unless otherwise noted. Organic acids were added as salts of potassium.

* i.e. vessels and tracheids in the xylem, sieve tubes, companion cells, and parenchyma in the phloem.

Except for the phloem of *V. vinifera* all the tissues, as dissected, contain some green cells but no effect of light on gas exchange was observed.

In all experiments the fresh weight of the tissues on cutting was used as the basis of calculation. The rates were expressed as $Q^F_{O_2}$, defined as oxygen uptake (in μl) per gram fresh weight of tissue per hour.

Alcohol-insoluble nitrogen was estimated by the method of McKenzie and Wallace (1954). Total sugars were estimated by a modified Somogyi method.

Mitochondria were isolated from *C. pepo* in 0·4M sucrose in Tris–"Versene" buffer at pH 7·2, using standard centrifugation procedures at 0–2°C. The fraction isolated at 12,000 *g* for 30 min was used in these experiments.

Each of the experiments reported below was repeated at least three times.

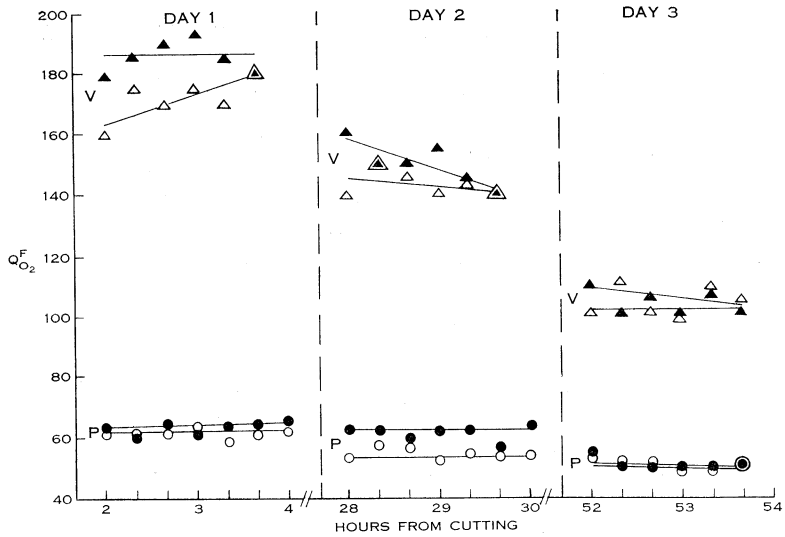

Fig 1.—*C. pepo:* $Q^F_{O_2}$ values for vascular (*V*) and parenchyma (*P*) tissues stored in water at 2, 28, and 52 hr from cutting.

III. Results

(*a*) C. pepo

(i) *Respiratory Levels.*—$Q^F_{O_2}$ values for vascular and parenchyma tissues over 2-hr periods at 3, 28, and 52 hr from cutting, are shown in Figure 1. The respiration rates fell with time, the decreases in the vascular tissue being greater than those of the parenchyma. The initial rate of the vascular tissue was two to three times as high as that of the parenchyma. Similar drifts were observed in ten experiments. Assuming (1) that lignified cells have no significant respiratory activity, (2) that the xylem parenchyma and cambiform tissue, which account for about 5% of the living tissue of the bundle, makes only a small contribution to the respiratory activity of the vascular tissue (the rate is taken to be equal to that of the "normal" parenchyma), and (3) that the normal parenchyma included with the vascular tissue is similar to

the ground parenchyma with which it is continuous, it follows that the rate of respiration of the phloem tissue must be considerably higher than that of the ground parenchyma. In the above experiment the $Q^F_{O_2}$ is calculated as being 540 μl O_2/g fresh weight/hr, i.e. nine times higher than that of the ground tissue. In 10 experiments differences of from approximately 5- to 15-fold were observed (Duloy 1960).

Two questions arise, (1) is the pathway of the high respiration of the phloem different from that of the low respiration of the ground tissue? and (2) does the presence of sucrose, which is thought to be the dominant mobile carbohydrate, affect the respiration of the phloem tissue?

(ii) *Response to Sucrose.*—The R.Q. values of both tissues stored in water ranged from 1·10 to 1·14 and remained unchanged over several days, and up to 90% of the loss of carbohydrate from the tissues could be accounted for by respiration.

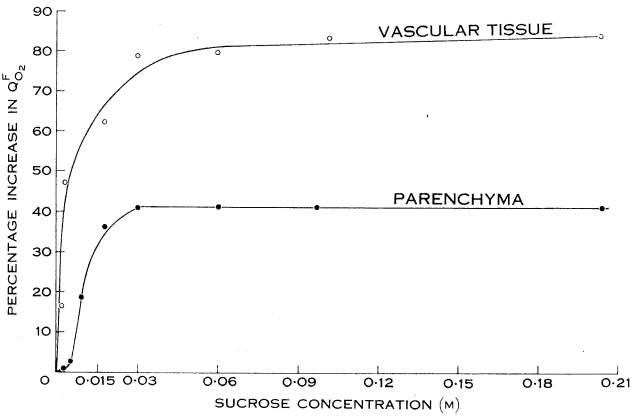

Fig. 2.—*C. pepo:* Percentage increases in $Q^F_{O_2}$ for vascular and parenchyma tissues with increasing concentrations of sucrose. Time from cutting 28 hr.

The respiration rates of both tissues increased with increasing concentrations of external sucrose up to 0·03M, no further increases being brought about by higher concentrations of sucrose (Fig. 2). The R.Q. values in the presence of 0·03M sucrose were almost identical with those in water, ranging from 1·10 to 1·22.

(iii) *Response to Intermediates of the Tricarboxylic Acid Cycle.*—Preliminary studies showed that stimulations in oxygen uptake of up to 25% were produced in both tissues by M/30 phosphate buffers at pH 5·0 and 6·5. Within this range, however, there appeared to be no effect of pH *per se* on the respiration of either tissue. From stimulations produced by the addition of M/30 KCl to tissue in unbuffered media, it was concluded that a salt effect due to potassium could account for about half of the stimulation produced by the buffer solutions. Since no further stimulations were produced by the addition of M/30 KCl to tissues in buffered solutions, it was concluded that a salt effect due to potassium did not make a significant contribution to the stimulations produced by the potassium salts of the intermediate acids.

As preliminary experiments showed that the respiration of both tissues attained steady rates and showed maximum response to treatment by day 2, experiments using intermediates and inhibitors were carried out with tissues 24–28 hr from cutting. Table 2 shows the response of the tissues to various intermediates of the tricarboxylic acid cycle. The addition of fumarate produced no response, but malate, succinate, and α-ketoglutarate increased the rate of oxygen uptake in both tissues, both in the presence and absence of sucrose, the effect being observed within 20 min of application. The R.Q. values rose only slightly, from 1·11–1·14 to 1·20–1·25, but an initial "gush" of CO_2 such as reported for other tissues (Turner and Hanly 1949) did not occur.

TABLE 2

C. PEPO: RESPIRATION OF VASCULAR AND PARENCHYMA TISSUES

Percentage increase in $Q^F_{O_2}$, over the rate in buffer or buffered sucrose, pH 5·0, produced by the addition of intermediates of the tricarboxylic acid cycle. Reaction mixtures contained: 0·25 g tissue, 4·0 ml M/30 phosphate buffer or buffered 0·03M sucrose, 0·45 ml intermediate, final concentration 0·05M. Time from cutting = 25 hr

	Malate	Succinate	α- Ketoglutarate
Vascular tissue in buffer	73	62	80
Vascular tissue in buffered sucrose	15	17	27
Parenchyma tissue in buffer	39	92	37
Parenchyma tissue in buffered sucrose	31	20	27

In all experiments with intermediates the respiration of the vascular tissue resembled that of the parenchyma, but had levels of activity two to three times greater. Values for the respiratory activity of the phloem calculated on the assumptions explained in the first section of the results, ranged from 550 to 1150 μl O_2/g fresh weight/hr.

The addition of 0·02M malonate produced a 70–95% inhibition in the oxygen uptake of both tissues, in the presence and absence of 0·03M sucrose, within 1 hr of application. In the presence of 0·05M succinate, malate, or α-ketoglutarate, however, malonate caused an inhibition of only 10–15% in the oxygen uptake.

(iv) *Terminal Oxidase.*—The addition of 10^{-3}M KCN caused an 80–95% inhibition in the oxygen uptake of both tissues within 20 min of its application. The succinate-stimulated respiration was similarly inhibited. Finally the succinate-stimulated oxygen uptake of mitochondrial fractions from both tissues was further stimulated by the addition of cytochrome *c* (Fig. 3) indicating that the tissues possess an active cytochrome oxidase system.

(v) *Response to 2,4-Dinitrophenol.*—It was found that 10^{-5}M dinitrophenol produced a 65% stimulation in the oxygen uptake of the tissues in water, and a 25% stimulation in the tissues in 0·03M sucrose, within 20 min from the time of application.

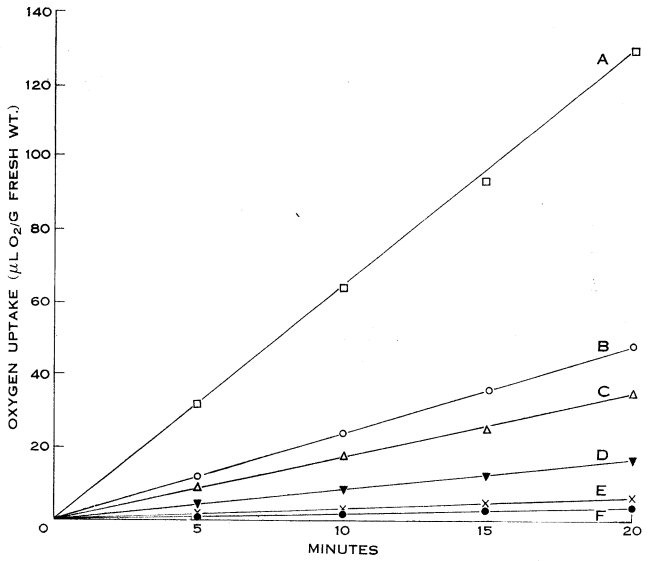

Fig. 3.—*C. pepo:* Oxidation of succinate and reduced cytochrome *c* by mitochondrial fractions from vascular and parenchyma tissues. The complete system contained: 1·5 ml mitochondrial suspension, 1·0 ml 1·6M sucrose, 0·4 ml 0·3M Tris–0·28M acetate buffer, pH 7·2, 0·5 ml water, 0·4 ml cytochrome *c* (60 μM). *A*, vascular tissue plus cytochrome *c* plus succinate; *B*, vascular tissue plus succinate; *C*, parenchyma tissue plus cytochrome *c* plus succinate; *D*, parenchyma tissue plus succinate; *E*, parenchyma tissue plus cytochrome *c;* *F*, vascular tissue plus cytochrome *c*.

(*b*) *Tissues from other Species*

Rates of oxygen consumption for tissues from *V. vinifera*, *V. virgatum*, and *A. graveolens* under various conditions are shown in Table 3. In calculating the rates of oxygen consumption in the phloem, the following assumptions were made:

(1) In *A. graveolens*, that the respiration rates of both the collenchymatous bundle cap of the vascular strand, and the xylem parenchyma, are equal to that of the cortical collenchyma.

(2) In *V. virgatum*, that the respiration rate of the collenchyma is 1·5 times as high as that of the parenchyma, i.e. that the rates have the same ratio as is found for these tissues in *A. graveolens*.

(3) In *V. vinifera*, that the fibres have no significant respiratory activity, and that the respiration rate of the ray parenchyma is equal to half that of the phloem conducting tissue.

So far as it was examined, the respiration of the phloem tissue of these species was qualitatively similar to that of the ground parenchyma tissues. In each species

TABLE 3

OXYGEN UPTAKE OF VASCULAR AND GROUND TISSUES OF THREE SPECIES

$Q^F_{O_2}$ values (μl O_2/g fresh wt. tissue/hr) in presence and absence of 0·03M sucrose, under various conditions. Time from cutting 28 hr. Solutions used: M/30 phosphate buffer, pH 5·0; 0·05M succinate; 10^{-5}M 2, 4-dinitrophenol (DNP)

Tissue	Water	Sucrose	Water + DNP	Sucrose + DNP	Buffer	Buffer + Sucrose	Buffer + Succinate	Buffer + Sucrose + Succinate
A. graveolens								
Parenchyma	30	45	40	95	40	83	65	90
Collenchyma	70	90	100	130	55	108	170	230
Vascular	450	460	500	660	400	400	480	490
Phloem*	1820	1810	1960	2590	1690	1540	1730	1600
V. virgatum								
Parenchyma	70	130	100	190	65	130	115	180
Collenchyma*	140	260	200	380	130	260	230	360
Vascular	260	440	500	540	290	500	490	520
Phloem*	850	1380	1780	1550	870	1643	1670	1503
V. vinifera								
Phloem segment	230	360			340	450	450	600
Ray parenthyma*	210	256			310	410	410	545
Phloem conducting tissue*	420	512			620	820	820	1090

* Calculated values.

the respiratory activity of the phloem, per gram fresh weight, was calculated as being 10–50 times higher than that of ground parenchyma tissue.

There being no surrounding ground tissue in *V. vinifera*, measurements were carried out only on phloem segments. The rates recorded were higher than those of the ground tissues of the other species, and of storage organs which have been investigated (see Spector 1956). The R.Q. values for *V. vinifera* at pH 5·0 were found to be 0·8 in the absence of sucrose, 1·2 in its presence, and 2·5 in the presence of succinate. The last value suggests that in this tissue the oxidation of the added succinate did not proceed via the normal catalytic cycle. No R.Q. measurements were carried out for *A. graveolens* or *V. virgatum*.

TABLE 4

ALCOHOL-INSOLUBLE NITROGEN CONTENT FOR TISSUES OF THREE OF THE SPECIES USED IN RESPIRATION STUDIES

Species	Nitrogen Content (mg/g fresh wt.)	
	Vascular Tissue	Parenchyma Tissue
C. pepo	1·07	0·35
A. graveolens	1·08	0·14
V. virgatum	1·96	0·50

(c) *Protein Nitrogen*

Protein (i.e. alcohol-insoluble) nitrogen were estimated for all species except *V. vinifera* (Table 4). The protein nitrogen content of the vascular tissue exceeded that of the parenchyma, so that in each species the respiration rate of the vascular tissue is found to be approximately equal to that of the parenchyma per unit weight of protein nitrogen.

IV. DISCUSSION

The results permit some general conclusions about the metabolism of the phloem tissue of *C. pepo*.

The observations (*a*) that the R.Q. values for both tissues in water are close to unity, and remain unchanged over long periods, (*b*) that approximately 90% of the loss of soluble carbohydrate from the tissues stored in water can be accounted for by respiration, (*c*) that the respiration rates are proportional to the external sucrose concentration up to 0·03M concentration, and (*d*) that the addition of sucrose produces little or no change in the R.Q. values, suggest that the primary substrate for respiration in both tissues is a soluble carbohydrate, and that this is in adequate supply, even in tissues without added sucrose, after several days.

The findings (i) that the oxygen uptake in both tissues is increased by the addition of α-ketoglutarate, succinate, and malate, (ii) that these increases are accom-

panied by only slight changes in R.Q., and (iii) that the malonate inhibition of oxygen uptake can be offset by succinate, α-ketoglutarate, and malate, together suggest that in both tissues respiration may proceed by a system generally similar to the tricarboxylic acid cycle.

Finally, from the findings (1) that the respiration of both tissues is extremely cyanide-sensitive, (2) that the succinate-stimulated respiration is similarly cyanide-sensitive, and (3) that the succinate-stimulated oxygen uptake of mitochondrial fractions from both tissues is further stimulated by the addition of cytochrome *c*, it seems likely that electron transfer in both tissues is mediated by the cytochrome oxidase system, to which is linked the succinic dehydrogenase system.

The stimulation of oxygen uptake by 2,4-dinitrophenol suggests that in both tissues some coupling exists between respiration and phosphorylation.

Collectively, the data indicate that the respiratory pathway in the phloem is identical with that in the parenchyma tissue, and generally similar to the pathways which have been found in other tissues, both plant and animal. That is, there is no evidence of a "unique" type of respiration either in the parenchyma tissue or in the phloem. Sucrose, which is regarded as the main mobile carbohydrate, is also respired by the phloem tissue, without inducing any alterations to the response of the tissue to respiratory intermediates and inhibitors, at least in isolated bundles. The results obtained for *V. vinifera*, *V. virgatum*, and *A. graveolens* support the conclusions reached for *C. pepo*.

Although the respiratory pathway seems to be identical in both vascular and parenchyma tissues in all four species, the respiratory activity of the phloem is apparently higher than that of the parenchyma, on a fresh weight basis, and may attain $Q^F_{O_2}$ values of almost 2000. These high values are consistent with the findings of Kursanov and Turkina (1952) and Kursanov, Turkina, and Dubenina (1953), who calculated $Q^F_{O_2}$ values of up to 5000 for the phloem of *Plantago*. Esau, Currier, and Cheadle (1957) have suggested that these high values may be overestimates because they were not corrected for the contribution of the xylem parenchyma and other living cells in the bundle. Even if such errors resulted in an overestimate of 100% the values would nevertheless remain very high as compared with storage tissue.

Recently Canny and Markus (1960) reported $Q^F_{CO_2}$ values of between 90 in water and 220 in sucrose for segments of phloem of *V. vinifera*, and concluded that phloem tissue does not have respiration rates significantly higher than those of ground or storage tissue. In contrast, in the present study $Q^F_{O_2}$ values of 230 in water to 450 in sucrose were recorded for phloem segments of *V. vinifera*, which after correcting for the possible contribution of the ray tissue gave calculated $Q^F_{O_2}$ values of the order of 1000 for the conducting tissue of the phloem. This estimate is dependent on the assumption that the respiration rate of the ray tissue is equal to half that of the phloem tissue.

There appear to be two groups of data concerning the respiration of the phloem, one indicating high rates, the other indicating low rates similar to those of parenchyma tissue. No explanation is offered of the differences between these groups of data. On the other hand an explanation of the high respiration rates calculated for

the phloem of three species described in this paper follows from a comparison of the protein nitrogen content of the tissues. Assuming protein nitrogen to be a measure of the protoplasmic content of a tissue, the higher activity of the phloem appears to be the result of its having more cytoplasm per unit volume of tissue than the parenchyma tissues.

The significance of the high respiratory activity of the phloem to the process of translocation, will be discussed in a later paper.

V. Acknowledgments

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