Organic Translocation

The green autotrophic plants can manufacture all their requisite amount of organic food-stuff within the body. The green tissues are the main site of their synthesis and the green cells are mainly confined to the leaves. All cells require organic food-stuff for normal growth and development. The non-green parts of the plants, therefore, must obtain the organic food-stuff from the leaves. Before the food-stuff actually reach the non-green parts, it must pass through intervening regions. *The movement of organic food-stuff within the plant from the site of synthesis to the site of utilization is known as* **translocation** or **conduction of solutes**.

14.1 Translocation of Organic Materials : It is evident from the earlier evidence that phloem (sieve tube) is the main pathway for the translocation of organic compounds. It is supported from ringing experiment (removal of tissues external to the xylem) demonstrated by a number of workers. Although it is undoubtedly proved that the channel of translocation is the phloem but the way in which the organic substances move in the phloem is a matter of great dispute and also a puzzling problem.

Among the earlier workers in this line may be mentioned the name of Hanstein (1860). He concluded from his experiment (by removing the extracambial tissues) that the food materials which are synthesized in the leaves and which are also necessary for the formation of adventitious roots, are translocated downwards through the extra cambial tissue i.e., probably through the sieve tubes.

This has been further supported by Mangham (1917) who showed that there is an active accumulation of starch above the ring together with the microchemical demonstration of sugars and organic nitrogenous compounds in the sieve tubes. These findings allowed the general acceptance of the idea that translocation of organic compounds occurs through the phloem.

These conclusion of the earlier workers have been heavily critised by Dixon (1923) while studing the accumulation of starch in the developing potato tuber along the cross sectional area of the sieve tubes. Dixon concluded that the sieve tube contents move at a rate of 50 cm per hour while the simple diffusion of sugar molecules takes place at a rate of 0.2 mm per day. These inevitably led Dixon to consider that the main channel of this movement is not the sieve tube but the xylem where the flow of organic compounds takes place like flow of water through a pipe.

It has been observed in later years that although Dixon's observation was very accurate still his conclusion regarding the path of the organic movement was incorrect. The exact path of conduction of organic substances has been found to be phloem by Mason and Maskell (1928) although in their ringing experiment they showed that a certain amount of leakage of metabolites from phloem to xylem takes place.

aphid was allowed to feed.

The transport through phloem takes place in symplastic way i.e., through the plasmalemma and living part of the plant, [for symplastic and apoplastic transport refer Chapter 5, article 5.17].

▶**14.3 Patterns of Phloem Transport :** Generally photosynthesis takes place in the leaves where carbohydrate are synthesised; in that sense leaves are considered as "source" from where phloem sap, moves in different directions to be utilized for metabolic activities, this is said to be "sink". So, the movement of the carbohydrate through phloem, require some general consideration as regard their transport.

(i) The phloem transport always takes place from source to sink. The mature leaves always act as a source or exporter while the half-mature leaves act as an importers or sink, in that case sap movement takes place into that half-mature leaves. Then when they fully mature, they switch over to the exporter stage and remain the same condition throughout their rest of lives.

(ii) From the upper mature leaves, the solutes move *upwardly* into the stem apex while from the lower mature leaves *downward* translocation to the roots takes place. Leaves in the middle protion are feed through *bidirectional* movement.

(iii) Radio isotopic experiments (refer article 14.2 in Chapter 14 for detail) reveals that the organic solutes move mainly through the phloem in a straight lines, very little *lateral* transport have been detected. Lateral transport takes place only when the source and sink lies on the opposite sides (Peel, 1966). Transport, from phloem to xylem and *vice versa* takes place slightly through leakage.

(iv) The pattern of source to sink transport may be changed by the removal of source or sink. As for example, if all upper mature leaves are removed, then lower leaves may export their solutes to the apex.

- **Bidirectional Movement :** Munch (1930) by removing girdles of bark, showed that phloem transport can takes place in both upward and downward directions simultaneously. In the upper growing regions where flowers, fruits, and young leaves are developing, the photosynthates move upwardly, while the downward translocation takes place to fed the roots, bulbs, tubers etc. In the growing condition, however, the stored food in the cotyledons are transported in an upward direction.

Biddulph and Cory (1960; '65) are of the view that bidirectional movement occurs in different layers or bundles of phloem. Many workers, however, are of opinion that bidirectional movement occurs in the same sieve tube (Eschrich, 1967 ; Canny, 1971).

▶**14.4 Mechanism of Phloem Transport :** The theories put forwarded to explain the mechanism of phloem ransport fall under three main groups, although none of them is entirely satisfactory.

(i) ***Activated Diffusion Hypothesis***—This theory, proposed by Mason and Phillis (1936), suggests that the rotoplasm within the sieve tube helps in hastening the diffusion of solutes and further the mode of activation equires respiratory energy and no experimental evidence in support of this, theory has boon forthcoming.

At present, it is evident that the sieve tube element does tiot posses any metabolic machinery responsible for driving the translocation process, except it helps only in the metabolic transfer which move solutes across the membranes.

(ii) ***Streaming of Protoplasm Hypothesis***—De Vries (1885) and more recently Curtis (1935) proposed that the streaming of the protoplasm within the phloem i.e., cyclosis is responsible for the transport of the solutes from one end to the other. Since the sieve tube elements are continuous from one tube to another, the theory accounts for the simultaneous movement of the solutes in both the directions (Fig. 14.1).

The best evidence for this theory would be the demonstration of simultaneous bidirectional movement within the smallest functional unit of conduction. This has recently been illustrated by Eschrich (1967) by feeding ^{14}C-urea to the upper leaf and the dye, fluorescein to the lower leaves of *Vicia faba*. Eschrich's idea regarding the phloem transport has been demonstrated in figure 14.2.

Advocates of the protoplasmic streaming hypothesis advance a great deal of evidence which suggests that translocation is an active process. For it has been shown that applications of certain respiratory inhibitors like KCN and azide, narcotics and low temperature (2-3°C) translocation of solutes may be stopped which affects the protoplasmic streaming rates.

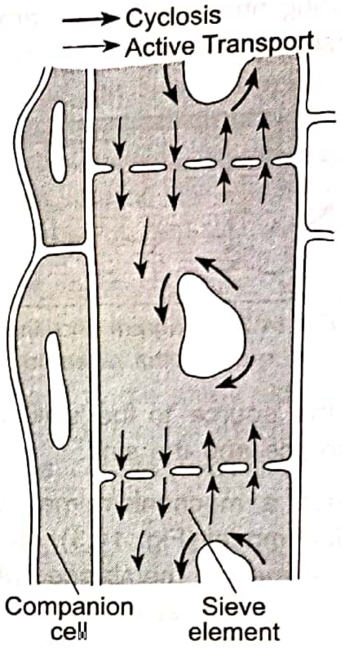

Fig 14.1 : Diagrammatic representation of protoplasmic streaming concept of translocation.

The main drawback of this theory is that although the streaming of cytoplasm is possible in young sieve elements but it is completely lacking in the mature one. Another weakness of this theory is the inadequency of the definite rates of protoplasmic streaming to correlate it with the known rate of solute transport through the phloem.

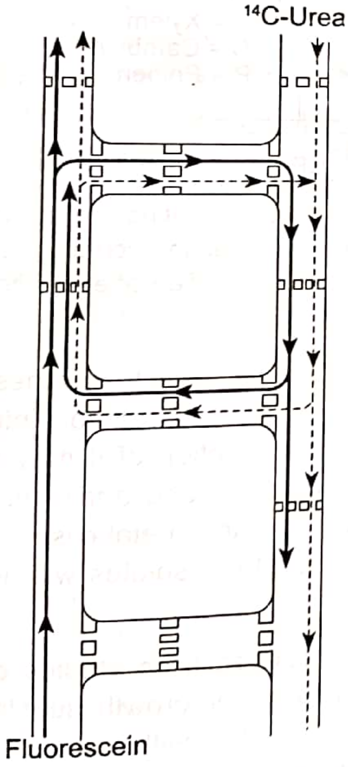

Fig 14.2 : Lateral transport and loop path to explain simultaneous bidirectional movement in a single sieve tube (modified after Eschrich, 1967).

According to Kamiya (1960), the maximum rate of protoplasmic streaming is found to be 5 cm/h whereas the rate of translocation can be upto 150 cm/h or more. Further, Schumacher's (1936 ; '37) observation on the movements of fluorescein strongly invalidates this theory. He showed the movements of fluorescein in a opposite direction to that in which the protoplasm itself was moving.

(iii) ***Transcellular Streaming Hypothesis :*** This theory is proposed by Canny (1962) and supported by Thaine (1962 ; 69). Since the solutes pass in the sieve tube in straight strands, Thaine suggested that there must have some continuous tubular strands across sieve tubes. Translocation through there strands takes place by a sort of peristaltic movement of the strands. According to Wooding (1969) these transcellular strands are actually microtubules, suited for the transport process. Electron microscope study, however, failed to spot transcellular strands.

The existence of P-proteins in sieve tubes (Fensom, 1972; Peel 1974) which are contractile in nature may help in the translocation. They claimed that these P-proteins played some role in pumping solution through the sieve pore. This hypothesis is also known as *contractile protein hypothesis*.

In 1979 Sabnis and Hart, however, reported that P-proteins are highly variable and not contractile in nature, as was proposed earlier.

(iv) ***Mass*** or ***Pressure Flow Hypothesis :*** This theory was initially postulated in 1860 by Hartig; but was more clearly formulated as a scientific theory by Munch in 1930.

In its original form, the theory included all the living cells of the plant, but now-a-days its functioning is thought to be restricted to the sieve tubes.

During photosynthesis, carbohydrates are produced and in the translocable form these carbohydrates exist as sucrose. Water which has ascended the stem in the xylem is absorbed by the leaf cells containing high concentration of sucrose as a result of osmotic forces and this in turn brings an increased hydrostatic (turgor) pressure in these cells. At the same time, a lowering of the concentration of sucrose in these regions where the assimilates are utilized for growth, storage and respiration, results in a lowered hydrostatic pressure Thus, the region of synthesis (green leaves) may be regarded as a **'source'** and the region of utilization as a **'sink'**. Because of the gradients of hydrostatic pressure, so created, there will tend to be a bulk or '*mass*' flow of solution and dissolved solutes from the 'source' to the 'sink' via phloem. So, this hypothesis is based on *turgor-pressure gradient* exists between the source and the sink.

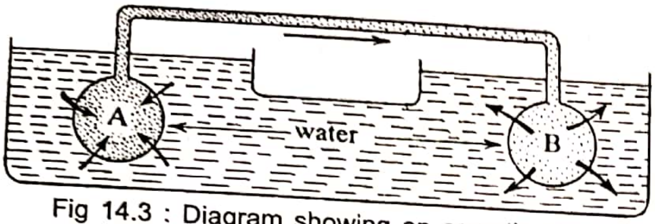

Fig 14.3 : Diagram showing an osmotic system to clarify the mass flow of solution.

Such a mechanism may be illustrated by means of a simple physical model (Fig. 14.3). As shown in the figure, the two spherical semi-permeable membranes (A and B) are connected with a tube to form a closed system.

Sphere A contains a solution of sugar, while B contains a weaker sugar solution or water and the whole set is placed in water. Osmosis will then cause an inflow of water in both the spheres, but a greater hydrostatic pressure will develop in A due to higher sugar concentration and hence water together with sugar solution will flow from A to B through the connecting tube. This will cause a greater diffusion pressure in the water in B than in the pure water in which the spheres are immersed. Water will, therefore, flow out from B to the surrounding medium and consequently there is a flow of sugar solution from A to B until the concentration of sugar in A and B is equal. To maintain a continuous system, sugar must bo added to A and should be equally removed from B.

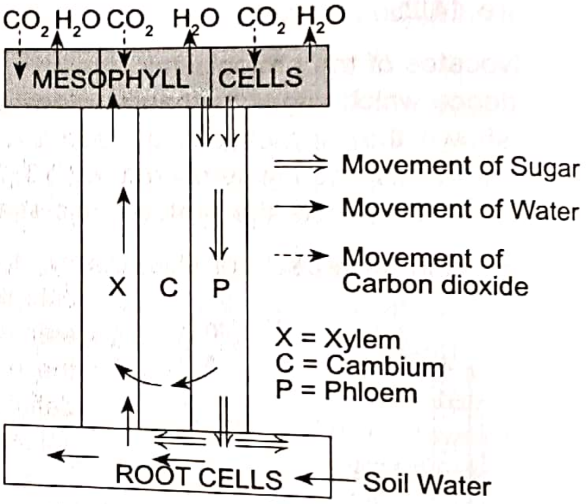

Fig 14.4 : Diagram illustrating the mechanism of solute translocation according to Munch's hypothesis (modified after Crafts, 1931).

As a result of photosynthesis, a greater osmotic concentration is maintained in the leaf due to synthesis of sugar; this causes a high turgor pressure in the mesophyll cells which allow some of the cell solution into the sieve tubes (Fig. 14.4). A major portion of the solutes moves down the sieve tube and a fraction of it may move laterally to cambium which is again forced into the xylem, join the ascending system of water and again move to the leaf cells. Major part of the solutes, however, reach the root where they are utilized in the metabolism or are converted to insoluble storage products, thus keeping a lower osmotic concentration therefore, solutes will always move from high to the low turgor pressure.

Perhaps the most convincing evidence **in support** of the mass flow theory has resulted from studies of the movement of extranneous substances in the phloem. It was observed that when viruses or growth substances were applied to illuminated leaves, they were rapidly translocated out of the leaves together with the assimilate stream in the phloem (Bennett, 1937; Rohrbaugh and Rice, 1949). However, when they are applied to shaded leaves, little or no translocation occurred. These observations strongly support the ideas that the sugars, produced during photosynthesis in the leaves, provided the turgor pressure gradients which are required for the operation of a mass flow. There is another explanation of the requirement for sugars, before translocation from the leaves can takes place. If the process of translocation involves the expenditure of metabolic energy, then the energy might be provided in the form of ATP as a result of the metabolic breakdown of the sugars during respiration. The strongest evidence of the requirement of ATP has provided by Rohrbaugh and Rice (1956). They showed that the translocation of ^{14}C labelled 2, 4-dichlorophenoxy acetic acid (2, 4-D) was severely reduced in plants which were deficient in phosphorus. Finally, sieve tubes contain solutes under a pressure, so that an injury in the phloem, results in the exudation of a solution rich in organic substances (Dixon, 1933; Crafts, 1936, '39; Huber, 1941).

- ***Electro-Osmosis*** and ***Mass flow Hypothesis :*** Two of the major objections to the Munch hypothesis are : (i) that it requires extensive turgor pressure to account for the flow through the pores of the sieve plates and (ii) that it is essentially a non-physiological theory, while the phloem tissues themselves have a high physiological activity. Both of these objections have been answered by Spanner (1958) in his **electro-osmotic theory**. In this theory, two types of mass flow was envisaged : a mechanical mass flow down the vacuole of the sieve element due to turgor gradient and an electrical or electro-osmotic mass flow through the pores of the sieve plates.

In explaining this hypothesis in physiological terms, it is considered that the pores of sieve plates are negatively charged containing more cations than anions. The water moves because of the electrical gradient and the sucrose molecules are dragged along with it. According to Spanner, the electrical gradient is maintained in the direction of flow, by a potassium pump. The K^+ ion moves through the pores and circulated back to the same side of the plate by an ATP- driven process in the membrane. (Fig 14.5). Perhaps the best evidence for this mechanism is demonstrated by Bowling (1968), who showed than a definite electrical potential difference does exist across the sieve plates of *Vitis vinifera*.

However, this hypothesis is questionable on the fact that Spanner's model accounts only for cation movement and nothing about anion movement where both ions moves through phloem. Further it is not applicable for the bidirectional movement.

- ***Objections to the Mass flow Concept :*** Opponents of the mass flow hypothesis have raised a number of objections as to its validity for all tissues. The theory demanded that all cells which supplied soluters to the flow (source) must have a higher turgor pressure than the cells which are receiving the flow (sink). Several workers observed that this was not always so. For example, it was seen that tissues such as cotyledons, ageing leaves and senescing petals often exported food to more turgid growing or storage tissues.

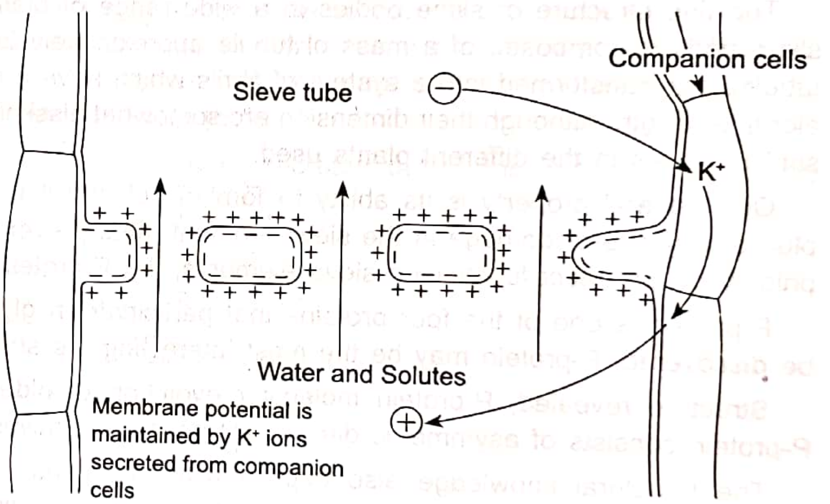

Fig 14.5 : Electro-osmotic flow of water and assimilates through the pores of sieve plate (modified after Spanner, 1958). Membrane potential is maintained by secretion of K^+ ions from companion cells.

Munch's original theory assumed that the sieve tubes were passive or dead and the protoplasm played no part in the transport mechanism. Since then it has, however, been shown that the translocation is associated with the metabolic activity in the phloem. Thus, for example, in the absence of oxygen or in the presence of certain respiratory inhibitors, like potassium cyanide (KCN) and 2, 4-dinitrophenol (DNP), translocation of sugars is invariably reduced or stopped altogether (Qureshi and Spanner, 1973). Furthermore, if some types of electro-osmotic force is operative at the sieve plates, metabolic activity might be required for the circulation of the charged ion about sieve pores.

A more serious objection to the mass flow theory resulted from the observation that the solutes could move simultaneously both upwards or downwards i.e., bidirectional. Instances of simultaneous bidirectional movement was demonstrated separately by Eschrich (1967) and Canny (1971).

Studies on the simultaneous movement of different substances revealed that they do not move at the same rate, i.e., the rate of water movement is 35 cm/h, while solutes have 72 cm/h (Catalado et al, 1972). This goes against the flow theory. For example, Vernon and Aronoff (1952) supplied $^{14}CO_2$ to the leaves of soybean plant and noted that the resulting radioactive sucrose had a higher rate of translocation in the phloem than the radioactive glucose and fructose which were also produced. Similar observations have been obtained by Biddulph and Cory (1957). Supporters of the mass flow idea have drawn an analogy of phloem transport with the solvent in the paper chromatography, where the solutes moves as a bulk flow with the solvent in the paper, but the different solutes undergo different degrees of adsorption and retention by the paper.

14.5 Translocation of Organic Materials :

P-Protein (Phloem Protein) :

By the best available techniques, the electron microscope revealed by either chemical fixation or rapid freezing and etching method that the contents of sieve cells show two highly variable features, the presence or absence of microfilaments of protein, called **P-protein** in various configurations and the filling of the sieve pores with these filaments. No positive actin reaction, such as binding with heavy meromyosin, have been detected. Sabins and Hart (1979) show that the P-protein is highly variable in its composition which bears no thickness to that of known contractile proteins.

It is actually a protein found in large amounts in the sap-conducting *sieve elements* of phloem tissue of plants. It takes various forms in the mature sieve element depending on plant species.

The small pores of sieve cells and the larger ones of sieve elements are traversed by strands of cytoplasm called P-protein. It is not known whether P-protein is active in transport or merely serves as a seal against leakage in case of injury.

The fine structure of slime bodies in a wide range of plants have been well investigated. In many cases the slime body is composed of a mass ol tubule approximately 200 Å in diameter. In maturing sieve elements these tubules are transformed into a system of fibrils which have a characteristic alternate light and dark staining bands along its length. Although their dimension arc somewhat dissimilar, the fibrils of the above studies probably represent similar stages in the different plants used.

One general property is its ability to form a gel and it functions as a puncture repair substances, forming a plug at any site of damage in the sieve element, thus preventing loss of food materials being translocated by the phloem. In an intact functioning sieve elements, the P-protein is mainly found lining the interioir wall.

P-protein is one of the four proteins that participate in glycine degradation. While its structure was the last to be discovered, P-protein may be the most interesting. Its structure is unique.

Structure revealed, P-protein molecular evolution unfolded to reveal the functional basis of its architecture. P-protein consists of asymmetric dimers, whereas all relatives of P-protein consists of symmetric dimers.

The structural knowledge also explain why mutations that create structural defects of P-protein decrease enzymatic activity, reduce glycine degradation and lead to disease.

Tubular and fibrillar forms of P-protein have been reported in sieve elements of wide range of higher vascular plants.

- **Mechanism of Phloem Transport :** The mechanism of phloem transport through the sieve tubes is mainly based on the internal organization of sieve tubes. Phloem tissue is mainly composed of the sieve elements, companion cells, phloem parenchyma and phloem fibres. Of all the above components, the sieve elements and companion cells are important for transport. The sieve elements are anucleated, elongated cells through which transport actually takes place. These elements are connected end to end through the pores of *sieve plates* forming a long tube like channels called *sieve tubes*. The companion cells connected to the sieve tubes have dense cytoplasm with small vacuoles. Here the nucleus is well defined. Sub-cellular organelles like mitochondria, dictyosomes and endoplasmic reticulum are abundant.

The ultrastructure of sieve tube shows some continuous smooth endoplasmic reticulum. Mitochondria are important sieve tube elements which are important in carrying out cellular respiration. In mature sieve elements plastids are present. Microfilament bundles are also reported in mature sieve elements. The presence of several types of fibrilar proteins with diameter 7-24 nm and molecular weight vary from 14,000 to 1,50,000 have also been reported. These proteins are referred to as phloem proteins or *P-proteins*. The pores of the sieve plates are blocked with these P-proteins. The occlusion of pores does not favour the pressure flow hypothesis. The other hypothesis, however, suggests that P-proteins play some kind of active role in pumping solution through the pores. It is however still a question, whether the sieve plate pores are open or occluded by P-proteins. So, more accurate observation regarding the ultrastructure ol sieve tubes is necessary for comming to a definite conclusion. However, the experiments of different workers can explain the following theories regarding photosynthates transport through the phloem.

●● **PHLOEM LOADING :** In majority of leaf phloem, sucrose concentration remains very high and therefore the osmotic potentiality of this region is also very negative. The mesophyll cells have osmotic potential of about –1.3 to –1.8 MPa (megapascal), whereas the leaf sieve element have an osmotic potential of about –2.0 to –3.0 MPa. So, the mesophyll cells act as 'source' cells from where the sucrose is transported and concentrated in the leaf phloem cells. *This process, the origin of the sucrose gradient, the pressure gradient and an active area is known as* ***phloem loading***. The smallest veins of leaves seem to be where most of the loading occurs. In 1977, using ^{14}C-radio isotope Fondy and Geiger showed that in case of *net-veined* leaves the smallest veins first to show the accumulation of ^{14}C-photosynthetate above the level in the surrounding mesophyll. In *parallel-venied* leaves, where small veins lie in groups between pairs of large veins connected sideways by small and sparse transverse veins, the specialization of the small veins for loading and the large ones for longitudinal movement, is specially well represented by Altus and Canny (1982).

The major event of translocation, the sucrose concentration step, must happen somewhere along the route from mesophyll cytoplasm to sieve tube lumen. Since the distance between mesophyll cells and sieve tubes is not more than 100 µm, there is no need of any pumping mechanism to raise the sucrose to a high chemical potential. Diffusion over such distances is a very rapid process, producing equilibrium of concentration in a few millisecond. There are two possible routes for the transport of sugar. The routes may be **apoplastic** i.e., cell walls outside the protoplast and **symplastic** i.e., cell to cell through plasmodesmata route.

There are ample evidence that sucrose is actively transported or secreted out of mesophyll cells into the apoplast of the minute veins from where the sucrose is absorbed actively into the companion cells. From the companion cells it is then pass hrough the symplast into the sieve elements (Geiger, 1975; and Giaquinta, 1980).

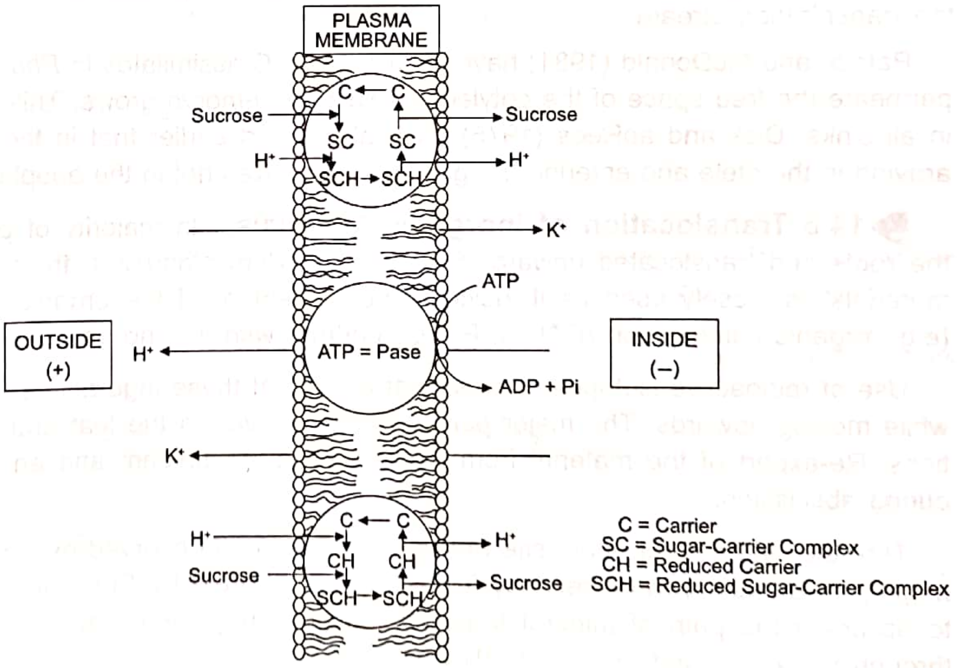

Fig 14.6 : A diagrammatic representation for active sucrose loading across plasma membrane into sieve elements. ATPase actively pumps out protons in exchange of K⁺. The protons return to the inside along with sucrose through a symport carrier system.

The whole process of secretion of sucrose from the mesophyll cells into apoplast lid subsequent loading of sieve companion cells and then to the sieve tubes are all active processes and the whole process occurs through the *co-transport* process.

There is little disagreement about the probable mechanism by which the sucrose accumulated across the boundary, wherever that boundary may be. All follow in adopting Mitchell's scheme for the translocation of energy of ATP by vectorial ATPase membranes coupled to the transport of protons across the membrane (Hinkle and McCarty, 1978; Nicholls, 1982). The energy for sucrose accumulation against a gradient chemical potential is generated by the extrusion of protons (H^+) at the expense of ATP. As the protons return across the membrane, a "symport" carrier system specific for sucrose accumulates sucrose molecules within the space. Such *sucrose-proton co-transport pumps* have been found in the cotyledons of *Ricinus* endosperm during germination (Komor et al, 1980). This type of transport is stimulated by fusicoccin and inhibited by a number of substances like dinitrophenol (DNP), valinomycin and *p*-chloromercuribenzene sulfonic acid. The various experimental evidences indicate that sucrose produced in photosynthesis must enter apoplast before being loaded into the phloem. From the apoplast, sucrose is actively loaded into he companion cells and then to the sieve elements.

The active sucrose loading across the plasmamembrane is represented in (Fig-14.6). The electro-genie proton pump not only push H^+ to one side of the membrane, but also exchange it for K^+ moving in the opposite direction to maintain electrical neutrality. For this reason, K^+ is universally present in the sieve tube exudates.

There are three main points to support the sucrose/proton co-transport process—

(i) the contents of sieve tube is known to have low proton (H^+) concentration and high potassium (K^+) concentration;

(ii) the apoplast at the loading sites is quite acidic in nature compared to the phloem sap and if the *p*H of the apoplast of a leaf is increased, sucrose uptake is inhibited;

(iii) the inhibitor which inhibits sucrose uptake in sugar beet leaves with the concomitant inhibition of proton secretion by the phloem minor veins.

Young leaves usually act as "sinks" and attract translocates from the mature leaves. When the companion cells of the minor veins of immature leaves develop phloem loading capacity change over this import mode to an export mode of transport (Giaquinta, 1983) and become sources instead of 'sinks'.

●● **PHLOEM UNLOADING :** As a result of increase accumulation of sucrose, more negative water potential facilitates osmosis from surrounding cells. Due to building up of turgor pressure, the photosynthates are moved to a "sink". At the downstream end, the sugars are actively moved out of the sieve tubes at the expense of metabolic energy by the companion cells. This active discharge process is known as **phloem unloading.** It causes the water potential of the sieve tube to become more negative and as a result water diffuses out of the sink to return to the transpiration stream.

Patrick and McDonald (1981) have shown that ^{14}C-assimilates in *Phaseolus* spread through the seed coats and permeate the free space of the cotyledons while the embryo grows. This simple pathway of unloading is not found in all sinks. Dick and apRees (1975) have also found earlier that in the growing tips of pea roots ^{14}C-assimilate arriving in the stele and entering the growing cortex was not in the apoplast but remained confined to the symplast.

▶14.6 Translocation of Inorganic Materials : In majority of plants the inorganic material absorbed by the roots and translocated upwards through the xylem along with the transpiration stream. The terms "inorganic materials" is loosely used as it includes the movement of the organic combination of the inorganic compounds (e.g., organic combination of N, S, P etc) together with the inorganic constituents.