Fig. 7.5: Figure explaining cation exchange theory (contact exchange theory).

MECHANISM OF MINERAL SALT ABSORPTION

Various theories proposed to explain the mechanism of mineral salt absorption can be placed under two broad categories:

(i) *Passive absorption.* It includes the theories of diffusion, ion exchange, Donnan equilibrium and Mass flow.

(ii) *Active absorption.* It includes theories related to carrier concepts such as cytochrome pump hypothesis, ATP theories, Protein lecithin etc.

PASSIVE ABSORPTION

Passive absorption may also be called physical absorption. It may be defined as the absorption of solute by cells according to ordinary laws of diffusion. Briggs and Roberston (1957) demonstrated the passive absorption of ions by root system in contact with soil colloids and root solution. They showed that the process is not affected by temperature and metabolic inhibitors and also that when a plant cell or tissues are transferred from a medium of low concentration to a medium of high concentration, there is a rapid uptake of ions. The direction of the initial uptake gets reversed if the tissues are transferred back to a medium of low concentration. Based on these findings various theories have been proposed to explain the mechanism of salt uptake.

1. Mass Flow Theory (Bulk Flow)

According to this theory ions are taken up by the roots along with mass flow of water under the influence of transpiration. Russel and Barber (1960) also supported this theory but raised a question whether the effect of transpiration is direct or indirect. Lopushinsky (1964) worked on this problem and studied the uptake of radioactive P^{32} and Ca^{45}. He found that an increase in the hydrostatic pressure (comparable to transpiration pull) increases ion uptake. So transpiration effect on salt absorption is direct. However, both mass flow theory and direct influence of transpiration have been challenged in view of recent researches. Both of these fail to explain salt accumulation against osmotic gradient.

2. Ion Exchange Theory

According to this theory ions from the external solution in which the tissue is immersed may exchange with the ions absorbed on the surface of the cell wall or membranes of the tissue. Just like ions exchanged between soil colloids and soil solution the cations may exchange with hydrogen ions and anions with hydroxyl ions adsorbed on the surface of the tissue and this ion exchange may allow greater absorption of ions from the external medium than could normally be accounted for by free diffusion. As hydrogen ions and hydroxyl ions are both readily available (adsorbed on the surface of the cell membrane), the cations and anions are exchanged freely. This process does not involve participation of the metabolic energy.

Ionic exchange phenomenon has been experimentally shown in excised barley roots with the use of radioactive K^+ in which case K^+ ions exchange place with non-radioactive K^+ ions. Likewise negatively charged Cl^- and Br^- are also exchanged without disturbing the electrical neutrality (Fig. 7.4). It is believed that a similar mechanism operates between soil solution and clay micelle which would allow for a greater absorption of ions from the external medium than could normally be accepted by free diffusion.

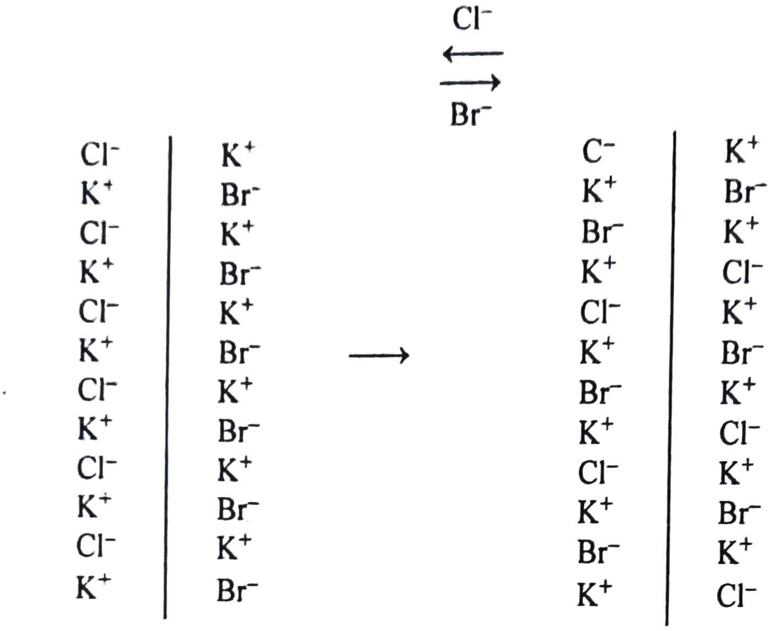
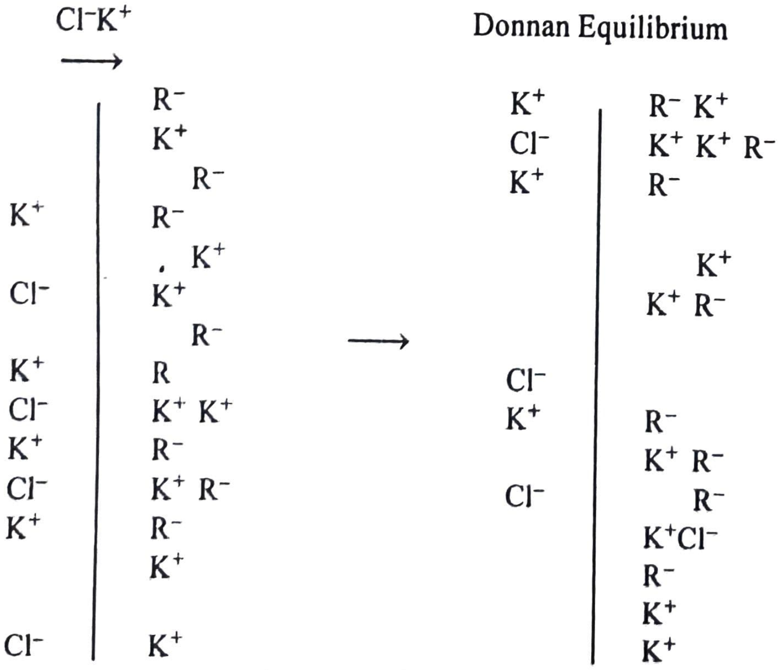


Fig. 7.4. Figure explaining ion exchange theory. Negatively Cl^- and Br^- are exchanged without. disturbing the electrical neutrality.

3. Donnan Equilibrium

The Donnan equilibrium theory accounts for the effect of fixed or indiffusable ions and assumes the cooperation of both electrical as well as diffusion phenomena (Donnan equilibrium) for the control of electrochemical equilibrium. This theory assumes that certain fixed or indiffusable ions (for which the cell membrane is impermeable) are present in a cell sap. In order to maintain an internal balance, such ions would require ions of other charge. Fig. 7.5 explains the basic concept of Donnan equilibrium.



$$4K^+ + 4Cl^- : 8R^- + 8K^+ \longrightarrow 3K^+ + 3Cl^- : 8R^- + 9K^+ + 1Cl^-$$

$$\frac{[Cl^-]_o}{[Cl^-]_i} = \frac{[K^+]_i}{[K^+]_o}$$

Fig. 7.5. Donnan equilibrium showing ion pair diffusion.

In this case ion exchange is prevented due to non-diffusable ions (R^-) inside the cell. At equilibrium, the above equation is maintained, *i.e.*, $\frac{Cl^-(\text{outside})}{Cl^-(\text{inside})} = \frac{K^+(\text{inside})}{K^+(\text{outside})}$ which means that ratio of number of Cl^- ions outside to inside the cell, $\frac{[Cl^-]_o}{[Cl^-]_i}$ is equal to the ratio of K^+ ions inside to that outside the cell, $\frac{[K^+]_i}{[K^+]_o}$.

When fixed anions are present on the inner side of the membrane, cations in addition to normal exchange would be absorbed to maintain the equilibrium thus increasing the cation concentration of the internal solution in comparison to external solution. The same type of mechanism involving additional absorption of anions would operate in case of fixed cations on the inner side of the membrane.

Objections to Passive Absorption Concept

Certain strong objections have been raised against passive absorption concept of salt uptake. A few of them are:

(*i*) In actual process, the rate of absorption of minerals is too rapid to be explained by passive absorption.

(*ii*) No theory of passive absorption adequately explains absorption and accumulation of salts or ions against the osmotic gradients (or against the laws of diffusion). However, cases of extra accumulation of K^+ ions within the cells (like 1000 times as against the surrounding medium) are now frequently known in *Nitella translucens, Chara australis* and *Hydrodictyon africanum* (Hober, 1945 and Raven, 1967).

(*iii*) It has been experimentally demonstrated that there is a close relationship between salt uptake and metabolic activities. This may be supported by the following examples:

(*a*) A quantitative relationship has been found between anion absorption and respiration.

(*b*) A close relationship between salt accumulation and respiration is found in all cases. Hopkins (1956) observed that salt accumulation is slowed, and even prevented completely, with the decrease in the oxygen content of the nutrient medium.

(*c*) The active phase of salt absorption is inhibited by the absence of oxygen, *i.e.* oxygen is required during salt uptake.

(*d*) There is a close relationship between metabolic activities and ability to absorb and accumulate solutes.

(*e*) The metabolic inhibitors influence the salt absorption. Lundegardh (1955) reported that salt uptake is inhibited by oxidase inhibitors, azides, carbon monoxides and cyanides (all metabolic inhibitors).

(*f*) Salt uptake has been found to stimulate and increase the rate of respiration. This increased respiration has been termed as *salt induced respiration.*

(*g*) Factors like pH, light, oxygen tension and growth affect the salt absorption suggesting that there is some essential role of metabolic activities in salt uptake.

These objections led scientists like Street, Lundegardh, Clark, Epstein etc. to support the active absorption concept of salt uptake. Various theories have been proposed. These are summarised as follows:

ACTIVE ABSORPTION

According to active absorption concept of salt uptake, it is believed that this process is supported by metabolic energy. There have been modifications from time to time to discuss the nature of participation of metabolic energy and that is why several theories have been proposed.

1. The Carrier Concept Theory

Observations of phenomena like selectivity, antagonism and synergism during salt uptake led Honert (1937) to propose the carrier concept theory of mineral salt absorption. According to this theory the ion transport process is carried out by means of carriers which may be organic molecules or vesicles. It is believed that ions undergo reversible binding with some constituents of outer space designated as carriers and pass through the impermeable boundary between outer space and inner space in the form of ion carrier complexes. On reaching the inner space they are again separated from carrier molecules. The direction of the movement of ion carrier complexes is from outer space to inner space only and ions released in the inner space cannot move out and thus are accumulated there.

This theory explains selectivity, abundance of selected ions in the membrane and their chemical affinities with the carrier molecules. Street (1962) explained the theory more beautifully with the help of a figure (Fig. 7.6). In the scheme, *X* and

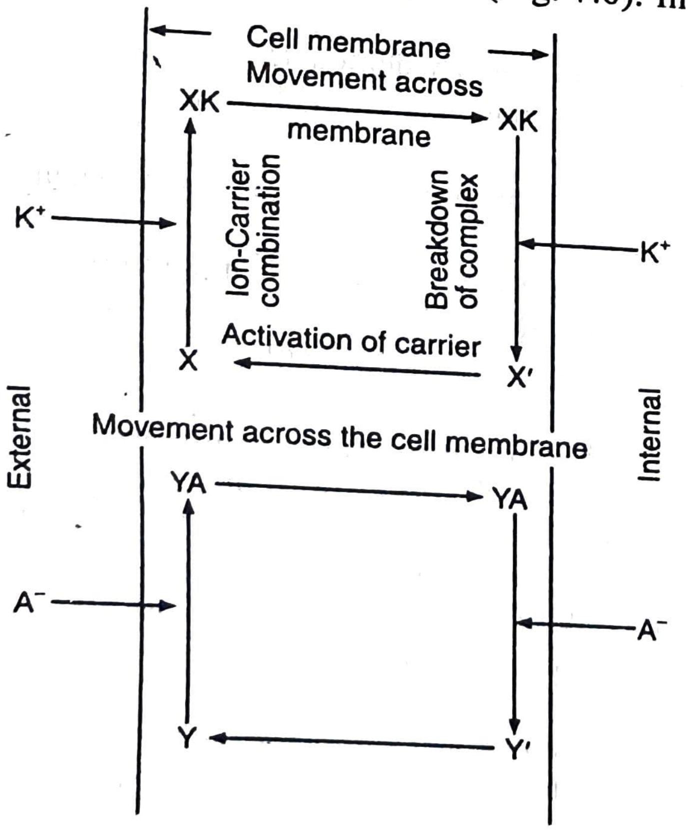


Fig. 7.6. Explanation of carrier mechanism of ion uptake by cells. (*X* and *Y* = carriers; *X′* and *Y′* = precursors; *XK* and *YA* = carrier ion complexes; K^+ = cation and A^- = anion) (after Street, 1952).

Y stand for carriers, *X'* and *Y'* for precursors of carriers. *XK* and *YA* for carrier ion complexes and K^+ and A^- for cation and anion, respectively. In the process carrier precursors *X'* and *Y'* are activated to form carriers *X* and *Y* which combine with ions to form ion carrier complex *XK* and *YA* for carrying them to inner space and releasing them over there. The metabolic energy is required in the process for regeneration of the carrier, ion carrier combination, transportation of ion carrier complex, breakdown of ion carrier complex and movement of carrier precursors back. The scheme accounts for both cations and anions but in the process separate carriers are involved and this is not accepted universally.

Sometimes, the word **translocator** is used in place of carriers. The **translocators** are specific large protein molecules. These can identify the mirror images of one another of the same compound, *e.g.* D-glucose which is metabolically important is absorbed whereas L-glucose which is not normally metabolised is not absorbed.

The carrier concept has three important supporting evidences:

(*i*) **Isotopic exchange.** Epstein (1956) pointed out that the fact that not only back diffusion but also isotopic exchange of the actively absorbed ions is prevented suggests a membrane highly impermeable to free ions. Leggett and Epstein (1956) in their experiments on absorption of sulphate by barley roots using labelled S^{35} found that free back diffusion or desorption of a portion of absorbed sulphate ions probably in the areas of the outer space is possible but a portion of sulphate ions which have been actively transported into the inner space cannot diffuse back during desorption and these ions are also not able to exchange for the stable isotope sulphate ions in the external solution.

(*ii*) **Saturation effects.** The carrier concept is also supported by the fact that when the concentration of the salt in the outer medium is increased, the rate of absorption also increases. But after a certain period, the rate of absorption becomes constant with no further increase. This level of maximum rate of absorption can be maintained for a relatively long period of time and this limit is reduced when the active carrier sites become saturated with the ions. Further increase in the rate is stopped because all the carrier sites remain occupied with ions and the constant rate is maintained because as soon as a carrier releases an ion in the inner space, it gets occupied by another ion for absorption.

(*iii*) **Specificity.** Selective absorption of ions by roots also supports the carrier concept. Ions are absorbed at different rates and have different levels of accumulation in the root tissue. This suggests the presence of specific carriers. Ions of dissimilar chemical behaviour have weak or non-existent specificity. Epstein and Hagen (1952) have shown that in movement—potassium, cesium, rubidium compete with each other for the same carrier site while sodium and lithium do not. This indicates that sodium and lithium have different carrier sites. Sodium has been recently found as an activator of plasma membrane ATPase. The presence of electrogenic sodium pump which causes entry of sugar and other metabolites in presence of ATPase-carrier has been proposed.

Many cells have *sodium-potassium exchange pumps* in the plasma membrane. It is a mechanism for transferring Na^+ and K^+ ions against their electrochemical gradient. The integral protein involved in this pump is a Na^+-K^+ dependent ATPase. For each molecule of ATP used, three Na^+ ions are pumped out and two K^+ ions are pumped in. The cell, thus, maintains a far higher Na concentration outside the cell than within.

Similarly, various ionic and molecular pumps remain present in protoplasmic membrane including a proton pump, a pump for K ions, another for Ca ions and still others for Cl and SO_4 ions, glucose, sucrose etc. Some pumps operate in reverse direction also.

2. Protein-Lecithin as Carrier

This theory believes in the participation of some amphoteric compounds as carrier with which both cations and anions can combine. Bennet Clark (1956) proposed that carrier can be a protein associated with phosphatide, *i.e.*, lecithin hence also called phosphatide cycle. In the transport of ions, the lecithin is synthesized and hydrolysed in a cyclic manner. Ions from outer space are picked up by lecithin to produce a lecithin ion complex which moves in the inner space and releases the ions on the hydrolysis of the complex. The process occurs in a cyclic manner as explained in the Fig. 7.7. During synthesis of lecithin, ATP are required.

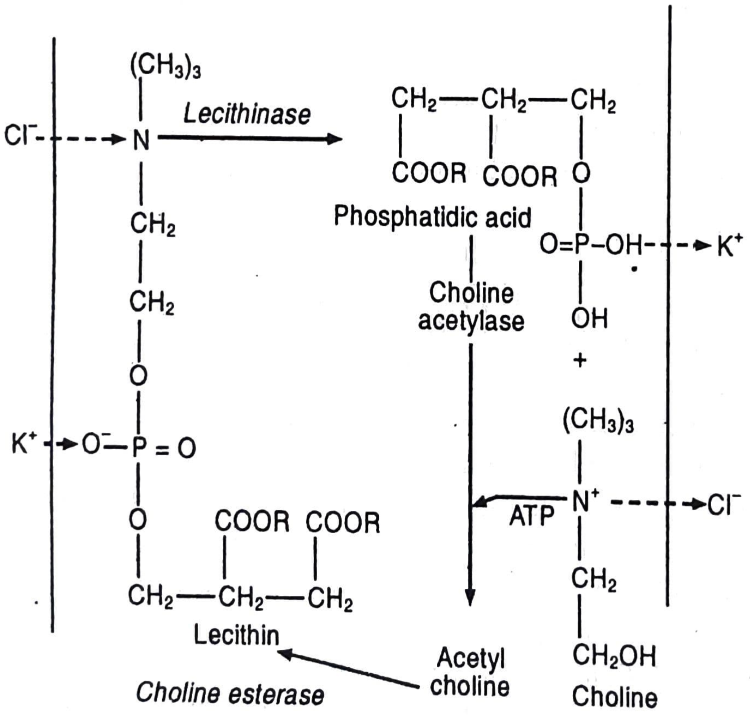


Fig. 7.7. Diagrammatic representation of the phosphatide cycle. Ions are picked up by lecithin from outer space and form lecithin ion complexes which on hydrolysis are released to inner space. Lecithin is then resynthesized. (Redrawn from Bennet-Clark, 1956).

3. Cytochrome-Pump Hypothesis

Lundegardh and Burstom (1933) claimed that a quantitative relationship exists between anion absorption and respiration. When a plant is transferred from water to salt solution, the rate of respiration increases. They termed this increase in respiration as *salt induced respiration.* later Lundegardh (1950) stated that anion absorption is independent of cation absorption and occurs by a different mechanism. Oxygen concentration gradient exists from the outer surface to the inner surface of a membrane favouring oxidation at the outer surface and reduction at the inner surface of a membrane. The actual transport of anions occurs through a cytochrome system.

According to Lundegardh, protons (H^+) and electrons (e^-) are produced on the inner surface as a result of dehydrogenase reaction. Via a cytochrome chain the electron moves outwards to be released to unite with protons and oxygen to form water, as a result of which the reduced iron of the cytochrome on the outer surface get oxidised by losing the electron as follows:

$$\{Fe^{++}/2A^-\} + A^- \rightarrow \{Fe^{+++}/3A^-\} + e^-$$

The oxidised iron of cytochrome on the inner surface gets reduced by taking an electron released from dehydrogenase reaction. The anions picked up by oxidised iron of the cytochrome on the outer surface are released on the inner side in the last step and in order to balance the potential difference caused due to accumulation of anions on the inner surface, the cations are absorbed passively.

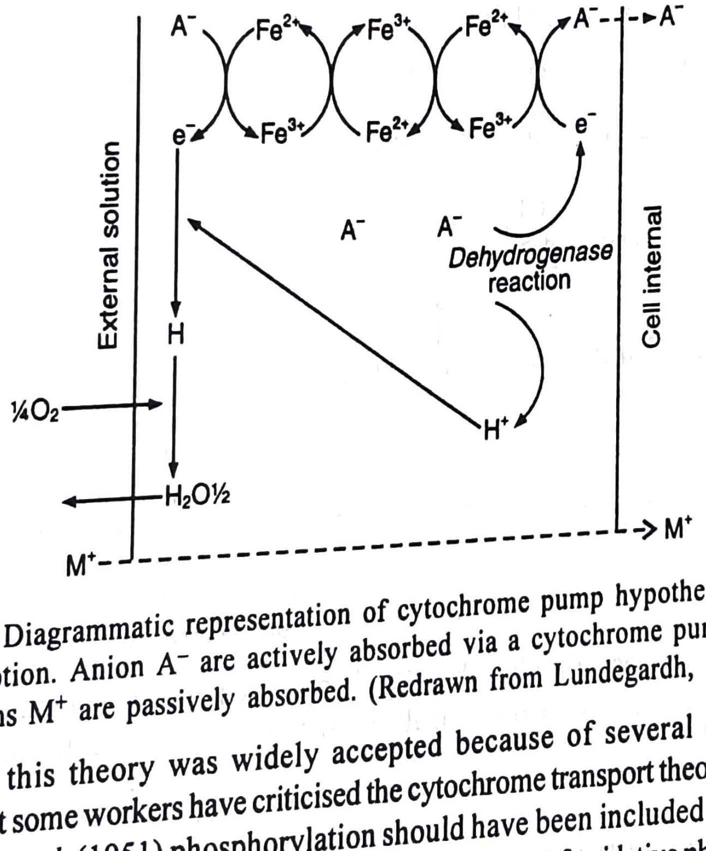


Fig. 7.8. Diagrammatic representation of cytochrome pump hypothesis on salt absorption. Anion A^- are actively absorbed via a cytochrome pump and cations M^+ are passively absorbed. (Redrawn from Lundegardh, 1950).

Though this theory was widely accepted because of several experimental evidences, yet some workers have criticised the cytochrome transport theory. According to Robertson *et al.* (1951) phosphorylation should have been included in any theory of ion accumulation as 2,4-dinitrophenol (DNP), an inhibitor of oxidative phosphorylation increases respiration but decreases salt absorption. The capability of anions only to stimulate respiration is also subjected to criticism as according to Handley and Overstreet (1955) sodium, potassium and ammonium ions also stimulate respiration. The absence of competition among anions for binding sites is objectionable when there is only one carrier for all anions.

This theory has several objections such as:

(*a*) It stands applicable only for anions and has no proper explanation for cation absorption.

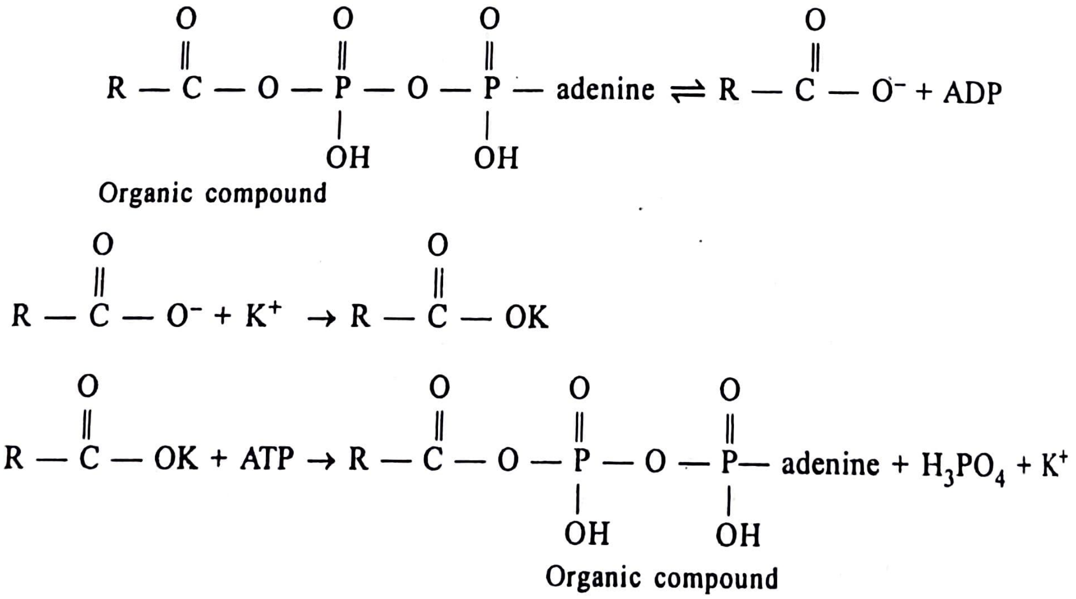
(*b*) It can't explain selectivity in ion absorption.

(*c*) It explains a common mechanism for all kinds of anion absorption which would not be able to explain the reason for different rates of absorption of ions of similar charge and to the competition incurred between different anions.

(*d*) In anaerobically respiring forms, such an elaborated electron transport system is absent.

4. ATP Theories

Specially for the cation uptake, Laties (1957) and Sutcliffe (1959) proposed ATP theories. According to them, ion uptake into the cell is energised by ATP. The energy from hydrolysis of ATP molecules can be made available to energise ion pumps through the action of enzymes. These enzymes have been found present in the cell membrane. The role of ATP in cation uptake can be of two kinds, *i.e.* by removal or by addition of phosphate group. In one case, organic compound is first phosphorylated which on dephosphorylation makes the organic compound capable of combining with the cation. The cation is released when phosphorylation occurs again. It may be explained as follows:



$$R-\overset{\overset{O}{\|}}{C}-O-\underset{\underset{OH}{|}}{\overset{\overset{O}{\|}}{P}}-O-\underset{\underset{OH}{|}}{\overset{\overset{O}{\|}}{P}}-\text{adenine} \rightleftharpoons R-\overset{\overset{O}{\|}}{C}-O^- + ADP$$

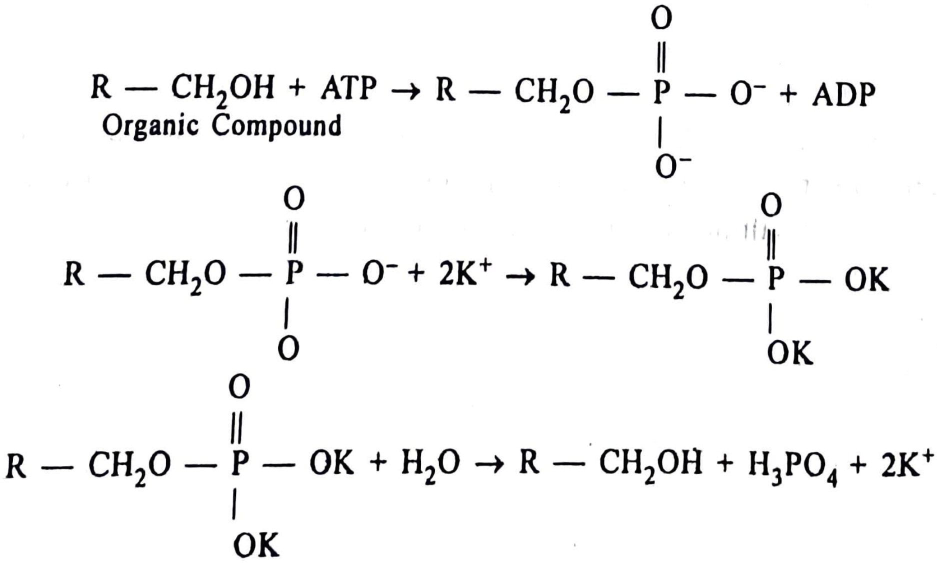
Organic compound

$$R-\overset{\overset{O}{\|}}{C}-O^- + K^+ \rightarrow R-\overset{\overset{O}{\|}}{C}-OK$$

$$R-\overset{\overset{O}{\|}}{C}-OK + ATP \rightarrow R-\overset{\overset{O}{\|}}{C}-O-\underset{\underset{OH}{|}}{\overset{\overset{O}{\|}}{P}}-O-\underset{\underset{OH}{|}}{\overset{\overset{O}{\|}}{P}}-\text{adenine} + H_3PO_4 + K^+$$

Organic compound

In the second case, the phosphorylated organic compound combines with cation and the cation is released on hydrolysis of the complex (dephosphorylation).



$$R-CH_2OH + ATP \rightarrow R-CH_2O-\underset{\underset{O^-}{|}}{\overset{\overset{O}{\|}}{P}}-O^- + ADP$$

Organic Compound

$$R-CH_2O-\underset{\underset{O}{|}}{\overset{\overset{O}{\|}}{P}}-O^- + 2K^+ \rightarrow R-CH_2O-\underset{\underset{OK}{|}}{\overset{\overset{O}{\|}}{P}}-OK$$

$$R-CH_2O-\underset{\underset{OK}{|}}{\overset{\overset{O}{\|}}{P}}-OK + H_2O \rightarrow R-CH_2OH + H_3PO_4 + 2K^+$$

Raven (1967) while working on algae such as *Nitella translucens* and *Hydrodictyon africanum*, has proved that cation uptake requires ATP while anion uptake is linked to electron transfer. The giant cells of these algae minimise the experimental difficulties and, thus, have provided conclusive information. These cells have sodium extrusion pumps and inwardly directed potassium and chloride pumps at the plasmalemma.

Conclusion

So, there are many theories to explain mechanism of mineral salt absorption but much remains to be concluded. Passive absorption concept has to face several criticisms while active absorption concepts have been widely supported. However, there are certain experimental evidences in support of passive absorption concept such as effect of transpiration on salt absorption and experiments of Lopushinksy (1960) which remain to be explained by the support of active absorption concept. On the other hand, evidences in the support of active absorption concept are quite high in number which could not be explained by supporters of passive absorption concept. Therefore, it is believed that one or both of these mechanisms may be involved in salt absorption. According to Wilkins (1969), the knowledge is in more primitive stage and there is need to fill up this gap by future investigators as far as possible.

FACTORS AFFECTING SALT ABSORPTION

Several factors affect salt absorption. Some of these are as follows:

(*i*) **Temperature.** Though within a narrow range, the increase in temperature increases both passive and active salt absorption processes and a lowering in temperature decreases them. This is because the same relationship exists between temperature and free diffusion involved in passive absorption and temperature and biochemical reactions involved in active salt absorption. Beyond a certain maximum limit of temperature salt absorption gets inhibited and stops because of denaturation of enzymes involved directly or indirectly in the processes.

(*ii*) **pH.** As the pH affects the availability of ions in the medium, it may indirectly affect absorption also. When sufficient ions are present in the medium, the effects of pH over a physiological range on salt absorption are not prominent. However, at pH values outside the physiological range damage to plant tissue and carriers inhibit salt absorption.

(*iii*) **Light.** As stomata when open allow more transpiration and increased mass flow and photosynthesis provides energy and oxygen for salt uptake, light indirectly affects the rate of salt absorption by affecting the opening and closing of stomata and the process of photosynthesis.

(*iv*) **Oxygen tension.** The deficiency of oxygen decreases salt absorption as the active phase of salt absorption is inhibited by the absence of oxygen.

(*v*) **Interaction.** The absorption of one ion may be influenced by the presence of another ion. The interaction appears to be primarily associated with the availability and specificity of binding sites on carriers. However, if enough binding sites on the carriers are present, the interaction will not be apparent.

(*vi*) **Growth.** Different types of growth affect salt absorption in different ways, *e.g.* growth involving increase in surface area, number of cells, synthesis of new binding sites or carriers and volumes of water uptake stimulate salt absorption. A good and heavily suberised root is unable to absorb salts. Vegetative growth and increased metabolic activity accompanied with more water uptake enhances salt absorption.