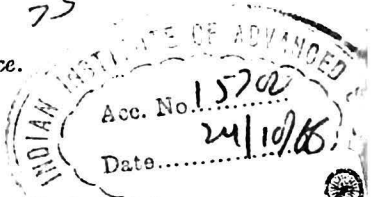


2 enps

11-10-56



CIRCULATION OF NUTRIENTS IN PLANTS

By D. M. BOSE

§ 1. INTRODUCTION

Life activities of organisms, whether consisting of single or mult cellular units, depend largely upon the interchange of materials across the semipermeable wall in which each cell of the organism is enclosed. Products of interchange are partly gaseous like intake of oxygen and elimination of carbon dioxide and vice versa and partly of molecules and molecular groups dissolved in aqueous medium. Except for the plant cells containing chlorophyll, the nutrients absorbed by such cells are mainly soluble sugar molecules, nitrogen and phosphorus-containing organic molecules, and mineral salts. Higher plants and animals are multicellular in which a certain amount of differentiation of function between cells has taken place. Between higher plants and animals the main differences are: (1) green plants are autotrophic, viz. they manufacture their own food material from simple inorganic molecules like water, CO₂ inorganic compounds containing mainly nitrogen, phosphorus, potassium, calcium, iron, etc. While animals live on food materials obtained from plants or from other animals, (2) land plants are usually rooted to the soil by means of the root system, while animals who have to seek their food are endowed with means of locomotion.

There are, however, some common functions present in both the two main branches of living nature. Each cell is bathed in a liquid medium, which carries the food materials which may also include carbon dioxide and oxygen in solution; these are absorbed across the semipermeable cell wall; across the same semipermeable walls are eliminated the waste products of cellular metabolism of which CO₂ is common. Each of these multicellular organisms contains a circulatory system; at certain places on this circulatory system, food materials and oxygen and CO₂ are absorbed and at other places the waste products are eliminated. There are therefore two main sources of supply and two or more main channels of eliminations. In animals the intake of food materials and water is mainly through the mouth; they are broken down into simpler units by process of digestion in the stomach and the intestines. These are absorbed across the intestinal wall by the blood-stream. The same blood-stream in its passage through the lungs absorbs oxygen and thus enriched it is made to circulate by the pumping activity of the heart through all the body tissues. Across the semipermeable lining of each cell, absorption of nutrient from and elimination of waste products to the blood-stream takes place. The waste products of the blood-stream are eliminated across the lungs, skin, liver and kidney. The whole process of circulation of nutrients and the elimination of waste products by the blood-stream is fairly well understood—the mechanism of circulation as the pumping activity of the heart, the energy required for the purpose is of chemical nature, viz. breakdown of carbohydrates, fats and proteins with the elimination of CO₂, water, urea, etc. The process of absorption and elimination across the semipermeable cellular membrane, the absorption of O₂ and elimination of CO₂ from the blood-stream, the absorption of water and nutrient salt in plant cells and elimination of waste



products across the kidney cells are not very clearly understood; detailed investigations are still necessary.

§ 1.1. *Mechanism of transference of materials.*—These may be due to one or several of the following processes:—

(a) The cell wall is permeable to certain substances; the transference of water and salts is then due to simple diffusion process, from regions of higher to that of lower solute concentrations; (b) the cell wall is semi-permeable to some of the solutes in the aqueous solution—e.g. if the cell contains a substance to which it is not permeable, e.g. sugar in solution, then water will enter through the semipermeable cell membrane till the osmotic pressure inside the cell wall is sufficient to stop further penetration of water. In the reverse case if the cell is immersed in a concentrated sugar solution the water will flow out of the cell and its enclosed vacuole and we obtain the phenomenon of plasmolysis; (c) in addition to the case of selective impermeability of certain membranes to molecules of sugar, etc., there are other instances of solute concentration gradients existing across plant and animal cellular membranes to which such membranes are normally permeable.

In certain large algal cells like fresh-water *Nitella* and the salt-water *Valonia*, the concentration of potassium in vacuolar sap is several times larger than that in the outer fluid medium, while the reverse is the case with the concentration of sodium. This condition has been found to hold for a large number of plant and animal cells.

§ 1.2. *Donnan equilibrium.*—When a membrane separates two solutions on one side of which contains a salt, both of whose anion and cation components like Cl^- and Na^+ can easily pass through the membrane, and on the other side there is salt with a common ion (say Cl^-) which can pass through the membrane while the cation is a heavy colloidal particle, to which the membrane is not permeable; this will as a consequence markedly influence the distribution of ions on the two sides. If, for example, we have a sodium salt of a protein on one side ($\text{Na}^+ \text{X}^-$) of concentration C_1 where Na^+ easily passes through the membrane, but not the protein ion X^- and on the other side a salt like $\text{Na}^+ \text{Cl}^-$ of concentration C_2 , both of whose components Na^+ , Cl^- can easily pass through the membrane, then the concentration of Na^+ will, in the equilibrium condition, be markedly different on the two sides of the membrane.

(a) In the initial state

	(1)		(2)	
	Membrane			
Ions	X^-	Na^+	Na^+	Cl^-
Concentration	C_1	C_1	C_2	C_2

After equilibrium has been reached, a certain concentration of NaCl will pass from the right hand side to the left, to satisfy the equilibrium condition

$$[\text{Na}^+]_1 [\text{Cl}^-]_1 = [\text{Na}^+]_2 [\text{Cl}^-]_2 \quad \dots \quad (1)$$

There is then a new distribution of ions on the two sides of the membranes.

(b) In the final state

(1) (2)
Membrane

Ions	X- Cl- Na+	Na+ Cl-
Concentration	C_1 C C_1+C	C_2-C C_2-C

From (1) it follows $(C_1+C)C = (C_2-C)^2$ i.e. the ratio of concentration of Na^+ on the two sides will be

$$\frac{C_1+C}{C_2-C} \text{ and of } \text{Cl}^- \frac{C}{C_2-C}$$

In this way a difference of concentration of the common permeable ion can be substantially altered.

A number of observations made on the salt content of vacuolar sap in large algal cells has shown that the latter is primarily a salt solution and the ions are neither held by to any appreciable extent in an adsorbed state, nor precipitated out in the form of insoluble compounds. The electrical conductivity of the sap was about what would be expected on the assumption that the total salt present in the sap was in an ionized form. The distribution of ions could not be explained in its major aspect in terms of Donnan equilibrium, which comes in the play when ions of one sign of charge cannot pass through a membrane (Hoagland, 1944; Osterhout, 1936).

The simultaneous absorption of anion and cation against concentration gradient has been found to be a feature of many other types of plant cells including those of storage tissues, leaves of water plants and root systems. The mechanism of such ion transport against a concentration gradient as due to consumption of chemical potential energy will be dealt with later.

§ 2. CIRCULATION OF NUTRIENTS IN PLANT SYSTEMS

§ 2.1. *In the higher plants there are two sources from which the plants receive their nutrients.*—(a) The root system by means of which the plants absorb water along with dissolved gases and salts. These inorganic nutrients must be brought into contact with each one of the root cells in contact with the outer medium. Recent investigations have shown that the growing root cells can also absorb CO_2 and amino-acids produced in the soil by the metabolic activities of soil micro-organisms (Kursanov, 1956).

(b) The mesophyll cells in the plant leaf system containing chlorophyll molecules are the seat of very important activities, due to which in these cells soluble carbohydrate molecules (hexose, glucose, etc.) are synthesized; their metabolic breakdown provides the energy for the synthesis of amino-acids, peptide bodies and from such soluble simple molecules plant cells, including the chlorophyll-containing cells, can synthesize many complex molecules, starch, proteins, nucleoproteins, enzymes, lipid bodies necessary for the structure and functions of the parenchymas as well as of differentiated cells.

(c) Each plant has to be supplied with mechanism for gas exchange. Intake of oxygen and elimination of CO_2 are necessary for each metabolizing cell. In addition to it, in the chlorophyll-containing cell and epidermal root cells CO_2 is absorbed and oxygen is given out during the process of carbohydrate synthesis.

(d) Recent investigations using isotopes of some common elements like carbon (radioactive C^{14}), nitrogen (heavy isotope N^{15}), phosphorus (radioactive P^{32}) have shown that the mesophyll cells and the growing root cells have some common activities.

(i) Both can absorb CO_2 and inorganic nitrogen compound and synthesize amino-acids, peptides and proteins.

Energy required for the synthesis of such endoergic molecules is supplied by the phosphorylation of carbohydrate breakdown products. In mesophyll cells soluble hexose molecules are products of photosynthesis, while in the growing root cells the soluble hexose molecules are translocated mainly from the mesophyll leaf cells.

(ii) Recently it has been found that deficiency in the supply through the roots of adequate amount of nitrogen and phosphorous compounds could be supplemented by means of foliar spraying methods.

§ 2.1.1. *Elimination of waste materials of plant metabolism.*—The mechanism used by the plant for elimination of surplus and waste materials is not so well known as in the case of the animal organisms.

Only a portion of the water absorbed by a plant through its root system is utilized in the process of photosynthesis, for cell metabolism and for cell multiplication. The bulk of the water absorbed by the root system is eliminated by transpiration from the leaf system of plants.

It is also known that, in certain instances, the excess of salts absorbed by a plant not used up for cellular metabolism and multiplication is returned to the soil. What happens to other waste products of plant metabolism is not known definitely, e.g. there is some speculation about the utility to the plants of the alkaloids produced by them, whether it is a waste product or not.

We shall not deal here with the mechanism of gaseous exchange in plants, as our main interest is in the mechanism of circulation of liquid nutrients in plant systems.

§ 2.2. *Channels for circulation of nutrient materials: anatomical structure.*—Having said something about the sources and sinks of the metabolism which are circulated in the plant system, we shall now say something about the channels in the plant structure through which the liquid nutrients are circulated as well as about the source of energy required for such circulation. Not much is known definitely about these two problems, many theories have been put forward, not one of which can be said to be able to account for all the known facts.

As we have said earlier the root and the leaf system are the two terminal organs which supply the minerals and the organic nutrients to the different plant parts, also each plant cell in the root, the stem, and the leaf system should have access to both inorganic as well as to organic nutrients. In each plant there are two vascular systems, the xylem and the phloem, of which the xylem is believed to be the channel for circulation of inorganic nutrient and the phloem of organic nutrient. We shall describe briefly the anatomical structure and location of these two principal conduction channels in the plant. This account is based upon Sinnott and Dunn's Botany (1955).

§ 2.2.1. *Structure of the root.*—Absorption of water and inorganic nutrient materials take place only in the younger portion of the root. The

latter can be divided into three zones: (a) the lowest the root cap, (b) the growth zone, and (c) the root hair zone. Each root hair is an elongated projection growing out from one of the surface cells of the root, its vacuole and lining of the cytoplasm being continuous with those of the root cell of which it forms part. It is across the extensive root hair surfaces exposed to the soil that absorption of water and nutrient salt takes place chiefly.

Inside the outer single layer cells, to certain number of which in the root hair zone root hairs are attached, is the cortex consisting chiefly of thin-walled rounded cells. These carry water and dissolved substances from the root hairs to the conducting tissues in the cortex. The innermost layer of the cortex is the endodermis. The fibrovascular cylinder occupies the core of the root, furnishing mechanical strength as well as serving as a highway for conduction. As in the stem the two principal conducting channels are the xylem and the phloem.

§ 2.2.2. *Stem*.—In dicotyledonous plants the outermost layer is the bark, next to which lies the cortex. Beneath this is the tubular fibrovascular cylinder consisting of xylem within and phloem without. We are interested here only in the description of these two constituents of the fibrovascular bundles.

Phloem.—The outermost layer of the vascular cylinder, between the endodermis and the phloem, is known as the pericycle. The fibrovascular cylinder is composed of two major layers of tissues. On the outside is the phloem the function of which is to transport elaborated foodstuff, from one part of the plant to the other, especially from the regions of manufacture to those of storage or consumption. The cells concerned in this process are the sieve tubes which are elongated living cells with cellulose walls, unique in not having any nucleus. In the end wall between two sieve tubes occur small perforations or sieve pits which make possible a direct continuity of protoplasmic thread from one sieve tube to the next, thus allowing materials to pass from cell to cell without diffusing through any cytoplasmic membrane. In the higher seed-bearing plants there is adjacent to each sieve tube a small companion cell with an abundance of cytoplasm and a nucleus.

Xylem.—The inner portion of the fibrovascular cylinder consists of the wood or xylem which gives both mechanical rigidity to the stem and also transports the stream of water and dissolved substances from the root to the stem. Most xylem cells are much elongated, parallel to the main axes of the stem, and their cellulose walls have become very thick and lignified. As soon as one of these cells is fully developed it dies and its protoplasmic content disappears so that only the thick woody wall remains. The pits, which occur in the wall and aid in the passage of water, are more complex than are the simple pits of parenchymous cells. In simpler kinds of wood one type of xylem cell is able to provide both necessary rigidity and conductive capacity and are known as the tracheids. Parenchymous cells, i.e. non-differentiated tissue cells, also occur amongst the lignified cells and may be elongated vertically. Other parenchymous cells are elongated at right angle to the axes of the stem and occur among the woody cells in horizontal bands or ribbons running through the xylem along radii of the stem. These structures are known as the wood rays and in somewhat modified form extend also into the phloem. They carry on horizontal transfer of materials in the stem and are of particular importance as centres of food storage.

A narrow layer of thin-walled cells, the cambium, separates the wood from the phloem; through cell divisions new cells are added to the outside

of the wood and the inside of the phloem, and in this way the thickness of the stem is increased.

We supplement the above description with a quotation from Miller's *Plant Physiology* (p. 849, 2nd edition).

'In this general description of the conducting elements of the xylem it should be noted that these tracheids and vessels which have lost their protoplasmic contents are yet in contact in some parts of the cell surface with one or more living cells. Abundant pits are also present in these contact areas. The living cells with which the non-living ones are in contact may be either the xylem parenchyma or xylem rays or both these elements.'

§ 2.2.3. *Leaf structure.*—At each node on the stem, a small but complete segment or series of segments of the fibrovascular system separates from the rest and passes through the cortex into the base of the petioles of a leaf. The internal structure of a leaf contains three kinds of tissues, the epidermis the protective layer, mesophyll cells the major substance of the leaf, and the veins, each of which is a fibrovascular bundle—channels by which the leaf tissue communicate with the rest of the plant. The tiny vein leads are in intimate contact with the cells of the mesophyll. The fibrovascular bundle is made of two kinds of tissues, the xylem which distributes the water and dissolved substances brought up through the stem from the root, and the phloem which collects from the mesophyll cells the foodstuff manufactured there and conveys them to the phloem of the stem and thus to the other parts of the plant.

For the successful activity of the leaf, particularly in the process of food manufacture, an interchange of gases between the inner tissues and the atmosphere is necessary. This is accomplished by the presence in the leaf epidermis of many minute openings, the stomata. Each stoma has a slit-like pore caused by the separation of two adjacent specialized epidermal cells of the stomata.

Stomata are of great physiological importance since they regulate the exchange of gases between the air and the leaf. The guard cells are so constructed that, when plump and turgid with water, they tend to pull apart, and thus widen the opening or pore between these. On becoming limp and partially collapsed, however, they spring together again and the pore is closed. The degree of stomatal opening thus fluctuates as the water contents of the guard cells rise and fall in response to changing internal and external conditions. The mesophyll consists of parenchymous tissues which are characteristically thin walled and green. The cytoplasm within the cells contains a large number of roundish bodies, the chloroplasts, which contain green chlorophyll; these are essential for the process of photosynthesis, by which from simple inorganic materials, carbon dioxide, and water, and with the energy derived from light, the carbohydrate and other compounds like protein, etc., are synthesized.

§ 2.3. *Rôle of leaf systems in the circulation of nutrients in the plant system.*—The water relations of plants are of the utmost importance and greatly influence their structures and activities. For salt absorption through the roots, the nutrients must be present in the form of an aqueous solution which ultimately reaches the leaf system, where water is an essential constituent of the process of photosynthesis. But only a small fraction of the water which enters the root hairs and passes upwards to the leaves takes part in the manufacture of carbohydrates. The remainder spreads through the cells of the spongy layer, evaporates from their moistened walls, and passes through the stomata as water vapour. This process is known as

transpiration. During the growing season a constant flow of water passes through the plant body.

The total quantity of water taken up through the roots usually amount to several hundred times the dry weight of the plant itself. One of the not clearly understood problems is the mechanism by which this amount of water is transported from the roots to the top of trees which may attain a height up to 300 feet. It is generally assumed that transpiration plays an important rôle in the process. This question we shall discuss later in more detail. The transpiration process depends on the rate of evaporation from the leaf surface and the latter depends upon the difference in the vapour pressure in the atmosphere surrounding the plant, from the saturation vapour pressure at the air temperature. Under conditions where transpiration is low but the absorption of water by the root system is still active, the excess of water may be exuded from water pores or hydathodes near the end of certain leaf veins, through which liquid water may be forced out in droplets, when it accumulates in excess. This process is known as *guttation*.

§ 2.4. *Ion absorption by epidermal root cells.*—We have discussed earlier two physical processes by which ions can pass across a permeable membrane separating two solutions with a common solvent. The first is simple diffusion when the ions move along the concentration gradient across the membrane. The second is the case of Donnan equilibrium when under certain conditions ions of one kind can move across a membrane producing a concentration gradient. We have, however, seen that in actual cases accumulation of say K^+ ion inside the cell vacuole takes place under conditions which are not in agreement with the assumption for a Donnan equilibrium.

§ 2.4.1. *Ion exchange.*—Before proceeding further we shall discuss some cases when exchange of ions of the same charge can take place between the surface of a cell and the surrounding medium. It has been found for example that radioactive potassium ions accumulated in barley roots can be replaced by exchange with stable potassium ions present in the surrounding medium. If, however, the root is placed in distilled water little loss of K^+ ion takes place. In general when from a medium K^+ ion is taken up by a root cell the latter will transfer a H^+ ion to the medium and for a Cl^- ion taken up from the medium a OH^- ion will be transferred.

§ 2.4.2. *Experimental observations on ion absorption.*—We describe below some relevant experimental observations which require interpretation on any theory which may be proposed to account for ion transport across cell surface.

(i) The transport of an ion like K^+ against a concentration gradient will not under normal condition continue indefinitely, but will reach a limiting stage, e.g. when passing from a low salt state to a high salt state of the absorbing tissue. The saturation effect is supposed to be the result of two opposing processes taking place simultaneously. In the first a chemical process of transport from the outside medium to inside the cell, and a reverse passive leakage (diffusion) from the higher ionic concentration inside to the lower concentration outside. This limiting condition will be delayed in certain cases, e.g. where there is a mechanism for utilization respectively for disposal of the absorbed ion; the former happens in growing tissues, when new cells are being formed, the second, when in roots the absorbed ions are transported inwards to the root xylem system, from where the solute can move along with transportation current through the tracheids to the leaf system.

(ii) One of the indispensable requirements for salt accumulation by excised barley roots (Hoagland, 1944) and of freshly cut potato discs (Steward and Street, 1947) is a supply of oxygen. In the latter case the dependence on cell metabolism is reflected but not stoichiometrically related to the aerobic production of CO_2 .

(iii) Under suitable conditions the rate of ion accumulation in plant tissues may be increased by supplying respiratory substrates externally to the absorbing materials.

It has for example been observed that salt absorption by excised barley root was related to the internal concentration of sugar, and when the carbohydrate content was lower, salt accumulation was increased by supplying them to the nutrient medium in which the roots were suspended. In potato discs addition of 0.1 M sucrose facilitates the absorption of ammonium ions by potato discs from a solution of ammonium chloride.

Steward and his collaborators (1947) have shown that the onset of ion absorption by fresh cut discs of potato tuber from an aerated mineral salt solution is related to the development of a potentiality for cell division. It was also established that absorption of mineral salts by roots occurs mostly near the apex, where growth is rapid. The influence of phosphate ion on salt accumulation and respiration of potato discs has been noticed by Steward and Street (1947). It has been suggested that salt absorption accompanying increased metabolic activities is associated with growth and cell division for which protein synthesis, an endoergic process, requires the supply of inorganic nitrogen compounds and of carbohydrate substrate. The transference of energy released during carbohydrate breakdown is mediated through the formation of intermediate phosphorylated compounds like hexose, phosphate, etc.

§ 2.4.3. *Metabolite synthesis in root cells.*—In a very interesting review on the application of radioactive isotopes to problems of agriculture and biology, A. L. Kursanov (1956) has reported on recent investigations which have taken place mainly in the U.S.S.R. on the above subject; similar investigations have also been reported from other countries. Using radioactive C-14 and employing the method of paper chromatography, the formation of amino-acids in the root system of plants has been observed by Kursanov *et al.*

When plants are grown in a soil-containing radioactive organic residues, the compounds due to microbial activity release radioactive carbonic acid (C^{14}O_2). At the same time, some of the soluble sugars formed by photosynthesis in the leaf are translocated along the phloem and on reaching the root penetrate into the root hairs. In the roots the sugar undergoes glycolytic breakdown resulting in the formation of pyruvic acid ($\text{C}_3\text{H}_6\text{O}_3$). The tagged CO_2 absorbed by the roots from the soil is converted by means of special enzymes to oxalacetic acid and enters into the Krebs's cycle. It can lead to the formation of stable organic acids. They can be partly translocated to the growing portion of the stem and be incorporated in the growing apices, leaves and fruits. Another part can interact with the inorganic nitrogen compound absorbed from the surrounding medium through the root hairs, and lead to the formation of amino-acids and peptides. These amino-acids can be utilized either for the formation of root proteins or can be translocated into other portions of the plants for protein synthesis.

These conclusions of Kursanov *et al.* (1956) make understandable the recent findings of E. S. Bollard (1953, 1956), on the occurrence of a large number of amino-acids in the tracheid sap obtained from a number of dicotyledonous, monocotyledonous and gymnosperm trees, as well as of

herbaceous species. Inorganic nitrogen in the form of nitrates occurs only in minute quantities in the sap, the rest of the nitrogen occurs as amino-acids and peptides. The author takes the view that a partial or complete assimilation of inorganic nitrogen must occur before it passes into transpiration system. The presence of organic phosphorous compounds has recently been detected in plant xylem sap.

§ 2.4.4. *Development of root pressure.*—The sap as it finds its way from the root system into the xylem vessels has been found to move under a pressure gradient to which the name root pressure has been given. This root pressure manifests itself when transpiration (due to evaporation of water from the leaves) is suppressed, by bleeding from cut stem or by guttation, viz. the extrusion of droplets of liquid from the tips of marginal leaves. The bleeding sap originating in the conducting vessels of the xylem has been found by several investigators to attain a relatively high concentration of inorganic solute, higher than their concentration in the external nutrient solution. This may be also true of the guttation fluid, which is often far from being pure water as sometimes assumed (Hoagland, 1944).

Hoagland has conducted experiments with young barley plants on the absorption and movement of salt and bleeding or guttation. If the roots are immersed in distilled water even though ample aeration is provided, guttation ceases or becomes slight in rate. If the roots are placed in dilute salt solution without aeration, guttation becomes negligible. If on the other hand the roots are immersed in dilute salt solution containing mobile ions and are well aerated and kept at a favourable temperature, rapid guttation continues for a long time in a humid atmosphere. Very little guttation was observed when the roots were exposed to a low temperature (5°C.). The exudation pressure from cut stem has been measured with a small number of plants. It has been found as high as 9 atmospheres by Boehm (1892), 6 atmospheres by Molisch (1902) in certain trees in Austria. For herbaceous plants grown under greenhouse conditions, root pressures have been found to range from 0.43 to 1.4 atmospheres. White has developed a method for measuring root pressure in excised tomato roots, where pressures up to 90 lb. were found to be developed. This pressure is sufficient to raise water up to 200 ft. height.

Hoagland reports the occurrence of periodicity in the development of root pressure in decapitated herbaceous or other similar young plants kept under a nearly constant environment, viz. in dark chambers with controlled temperature and humidity. Despite constancy of environment, the plants passed through 24 hours' cycle of variation in root pressure. A protoplasmic rhythm is suggested, which governs the cell activities affecting salt-water movement into or out of conducting system. It therefore appears that guttation and development of root pressure depend largely on physiological conditions and on cellular metabolism.

§ 2.4.5. *Movement of water.*—Before considering the mechanism underlying the translocation of solutes through the root system, which is accompanied by the development of root pressure, it is necessary to say something about the mechanism of movement of water through the root system. It appears that the movements of water and the solute through the plant system are largely independent of each other. The transport of the solute may be due to two causes, ordinary physical diffusion which follows the direction of concentration gradient and the other activated transport in which the solute moves against concentration gradient for which consumption of chemical energy is necessary.

The movement of water is generally a physical process of diffusion, whose tendency is to equalize solvent concentration on the two sides of a

It is further assumed that the protoplast AB is permeable to ZR and $R'A$ but not to the ions Z^+ , A^- , R^- , R^+ ; we have then a working model of the transport of ions from the outer medium to the vacuole. The energy released by the combination of H^+ and OH^- is supplied by substrate metabolism (for details see Overstreet and Jacobson, 1952).

§ 3.1.1. *Anion absorption*.—The most quantitative of such hypotheses is the model proposed by Lundegardh and Burstrom (1933, 1935). They observed that in the presence of mineral salts the rate of oxygen absorption by isolated wheat roots in culture is increased above the level which is attained in distilled water, by an amount which is directly proportional to the number of anions absorbed. No such exact relationship we found when the absorption of cation was studied; and on this account the stimulated component associated with respiration was termed 'anion respiration'. This was shown to differ from the basic or 'ground respiration' which takes place in distilled water. The former and not the latter is susceptible to cyanide poisoning. It was postulated therefore that the anion respiration may be mediated by a cyanide sensitive terminal oxidase system such as that involving cytochrome and cytochrome oxidase. If such an enzyme system is located at the boundary between two media of different redox potentials, then the transport of the anion may be effected. It is assumed that at the surface of a root or of a cell protoplast, the cytochrome will be mainly in the oxidized condition. The ferric ion of the heme group of the cytochrome system is capable of absorbing a certain number of anions from the external medium by electrostatic attraction. The cytochrome molecules are supposed to be in a state of thermal agitation so that their positions on the membrane are altered from time to time. In this way the cytochrome with its absorbed ions may be transported to a region of lower oxidation potential where the iron ions are reduced to the ferrous condition, and one anion is released for each atom of iron reduced. By this mechanism the transport of anion across a membrane which is impermeable to diffusion of free ions may be brought about.

The reduction of cytochrome again from ferrous to ferric state involves the acceptance of an electron in exchange for each anion released, so that in this system there is a transport of electrons in the opposite direction to that in which the anions are moving. The electrons which are transported may be derived from hydrogen atoms made available in dehydrogenase reactions of the Krebs' organic acid cycle, through the intermediary of flavoprotein. Transfer of electrons from hydrogen atoms to cytochrome results in the production of hydrogen ions. These may diffuse towards the outer surface of the membrane, along a diffusion gradient, where they are recombined with electrons from cytochrome at this point in the presence of molecular oxygen and the appropriate enzyme, with the production of water as the ultimate respiratory product.

§ 3.1.2. *Absorption of cations*.—Accumulation of anions by plant cells and tissues is usually associated with the absorption of an equivalent number of cations, since electrical neutrality of the system must be maintained. This absorption may take place along a potential gradient created by active anion absorption. There may also be metabolic processes other than anion absorption, which result in the production of negatively charged particles inside a cell and this can lead to cation absorption. The presence of reactions such as the production of organic acids from sugars may account for the absence of direct relationship between absorption of cations and anion respiration in wheat roots.

The theory proposed by Lundegardh and its variants proposed by others give reasonable models of carrier mechanism for the transport of

ions against concentration gradient in plant cells and tissues. We have mentioned before the observations of Steward and others on the influence of phosphate on salt accumulation. We shall not enter into details of the mechanism which has been proposed to account for the rôle of energy rich phosphate compounds in salt transport mechanism. It appears that Lundegardh's mechanism may only be a part of a more complex transport system. His hypothesis provides a satisfactory working model of transport of ions against concentration gradient based upon utilization of energy of substrate oxidation.

We have so far considered the absorption of salt by plant cell. It is not definitely known where the transport mechanism is located. One theory is that the latter is situated in the outer surface of the protoplast, and that the active absorption takes place in the protoplast from which the ions pass into the vacuole by diffusion along a concentration gradient.

It has been claimed by other investigators that in *Nitella* for example the concentration of ions in the vacuoles may exceed that in the protoplast or that in the outer medium, so that an active transport of ions from protoplast to the vacuoles may occur. Difficulty in interpretation is that the concentration of ions in the protoplast is not easy to calculate. Many consider on experimental evidence that the tonoplast is a relatively impermeable membrane.

§ 3.2. *Salt absorption by roots.*—The absorption of mineral salts by root system is more complicated than the accumulation of ions into single cells. The question has not been yet satisfactorily settled as to the seat of the motive power which drives the salt through the root system across the cortex into the conducting elements of the stele from where they are carried into the different parts of the plant. One possible view is that the concentration gradient due to accumulation of salt in the vacuole provides the motive power for diffusion of salt from the accumulating root cells to the conducting elements of the stele. The other view is that there are two distinct processes, one the movement of solutes into the vacuoles of each individual living cell of the tissues, and the other transport of solutes across the cortex into the conducting elements of the stele. Broyer (1950) claims to have shown that when the initial salt content of the root is high the ions move mainly into the xylem, whereas in 'low salt' roots transport is at first mostly into the vacuoles of the cortical cells. Salts in the vacuoles of the parenchyma cells may move into the stele when salts are not available to the plant in the external medium. Munch (1930) introduced the idea that the protoplasts of all living cells are continuous with one another through protoplasmic strands forming a single unit the 'symplast'.

There are several hypotheses of how the salt imbibed from the outer medium through the root hair cells finds its way ultimately into the xylem vessels. Of these we shall mention the one proposed by Lundegardh (1950) according to which there exists an oxidation reduction potential gradient across the root cortex, which according to his theory of salt absorption may account for the inward transport of mineral salts. He has established that the rate of bleeding of excised roots is only indirectly related to anion respiration and that the movement of ions into the stelar tissue represents leakage against a concentration gradient maintained by an active transport mechanism. There is an alternate hypothesis proposed by Arisz (1945) in which the cytochrome system which forms the basis of Lundegardh's transport theory is located in the mitochondria, a particulate body in which a multi-enzyme system associated with the *Kreb's* cycle is located. These mitochondria bodies are randomly carried about with the

protoplasmic streaming. They are seen to congregate at regions of rapid metabolism, e.g. round the nucleus, the vacuole and the outer boundary of the cell protoplast. It is suggested that the active ion secretion takes place first to the mitochondria which then can transport it to the plant vacuoles; alternately they can be carried along the symplast. At the boundaries of symplast with the xylem vessels the adsorbed salts may be transferred by leakage across a concentration gradient. In a subsequent section (§ 6.2.2) the part played by mitochondria in the transport of nutrients in protoplast as well as in phloem sieve tubes will be considered in some detail.

Due to the difficulty of determining the relative salt and other solute concentrations in the outer medium, in the vacuole, in the protoplast and in the xylem vessels, there are no means of definitely deciding between the validities of the different hypotheses proposed to account for the transport of solute across the root system of plants. Two well established facts are: concentration of salt in vacuoles and protoplast tissues by means of active transport, and the occurrence of root pressure in xylem vessels in the stem. Both of these observations are explainable on the basis of a theory of active transport of solute.

§ 4. *Translocation of root sap through the stem.*—The ascent of sap from the root system to the leaves under condition of high evaporation has been proposed to be accounted for by two main types of theories, vital and physical. The accounts given below are based upon Miller's review given in his book *Plant Physiology* (1938).

The authors of vital theories assume that the forces that are active in raising water in plants are manifested for the most part by the living cells of the root, stem, and leaves, although most of them consider that purely physical forces may assume a definite though a minor part. Those who propose physical theories contend that the major part of the forces concerned is purely physical in their nature and consider that their manifestations are identical or similar to that which would occur in a purely physical apparatus under like condition. Most of those who favour the physical theory of the ascent of sap agree that the living cells in plants play their part in the process, although these actions are of only minor importance. It would seem that the forces which are dominant may depend upon the conditions surrounding the plant. Thus under some condition, e.g. low evaporation, vital forces are the dominant ones, and physical forces might be exerting only slight influence. While under another set of physical conditions, e.g. of high evaporation, physical factors may dominate the process and overshadow the activities of living cells.

§ 4.1. *Physical theory.*—We shall consider first some evidence, that the major portion of the water movement from the root to the leaf system passes through the conducting elements in the xylem vessels in the stem, which are made up of cylindrical shaped vessels from which protoplasm has disappeared, and not through the bark which contains living parenchymous cells. Recent evidences in support of this are given by the investigations of Hoagland and Stout (1944). They used salt solutions containing radioactive isotopes of Na, K, P and Br as tracer, in studying the upward movement of salts in actively growing and transpiring willows and geranium plants, after absorption of the isotopes by the roots. A certain portion of the bark was isolated from the stem by means of paraffined paper. In the isolated strip of bark, the radioactive element moved in very slowly. Within a short period no significant amount of radioactivity could be detected in the central portion of the bark strip, while much larger amounts were present in the wood. However, when wood and bark

were in contact, radioactivity was rapidly transposed from wood to bark. The evidence is consistent with the view that the xylem is the path for rapid upward movement of salt, also there was a certain lateral translocation from wood to bark.

These and allied investigations do not throw any light on how purely physical force can be responsible for the upward movement of water in plants. The most systematic exposition of the physical theory is Dixon and Joly's cohesion theory of ascent of water in plants (1894). The theory assumes that water in the conducting tracts of the xylem exists in form of unbroken columns, and these columns are continuous both vertically and horizontally through cell walls. A continuous meshwork of water thus exists throughout the whole length of the stem, root and leaves. The terminals of the water meshwork are the menisci of the water in the sub-microscopical cavities of the cell walls of the epidermal cells of the root and the cells bordering on the inter-cellular space of the leaf or the epidermal cells. The theory assumes that water in the conducting tract is brought to a state of tension by the imbibitional forces in the evaporating cell walls of the leaf, and these water columns are able to withstand the tensile pull that is exerted upon them.

It may be mentioned here that the terms 'imbibition' and 'capillarity' are used by some writers more or less interchangeably. Here, however, capillarity is confined to the small but well defined lumina or tubes while imbibition is confined to interstices of the cell wall. The force of imbibition in the cell walls of the leaves can be enormous depending upon the degree of drying and is thought to be much more than ample to lift a column of water to the top of the tallest tree.

The result of transpiration of water from the terminal cells in the leaves creates a saturation deficit in these cells resulting in partial dehydration of the cytoplasm of the cell. This leads to an abstraction of water from the neighbouring cells. This disturbance in equilibrium is transmitted eventually to the water of the xylem elements of the leaf. A pull is thus set up ultimately on the water column in the vessels of the conducting xylem elements of the stem and root and then to the soil. Owing to the cohesive force of water, which can according to investigations reported by Dixon, Ursprung and Renner (*see* Miller, 1938) can stand up pull even when containing dissolved water, up to 300 atmospheres; the inhibitional force due to leaf evaporation could be transmitted as a tensile pull to the water menisci in the root hair cells. The following model of a capillary water column with its upper end A exposed to the atmosphere, and the lower end W separated from a plain surface of water by a concave meniscus, below which there is water vapour at saturation vapour pressure lying over a plain water surface at the ambient temperature T .

If the vapour pressure over the end A is the saturation one at temperature T , then according as the surface at A is convex or concave, water vapour will evaporate from or condense over surface A . Since it has to support the column of water of height AV , the meniscus at A is concave, water will only evaporate from A if there is a saturation deficit of sufficient amount in the atmosphere. When there is such evaporation from A the surface at W will become more concave, and water vapour from the plain surface of water will condense on the concave surface of W . Thus a transpiration current will be maintained due to purely physical conditions so long as the atmospheric vapour pressure at temperature T is less than the equilibrium vapour pressure over the curved surface A . This is the physical basis of Dixon and Joly's theory of transpiration of water.

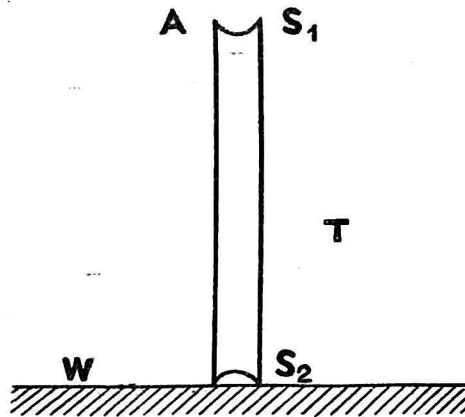


FIG. 2

AW —Capillary tube filled with water. S_1 and S_2 —Curved surfaces exposed to atmosphere A and water surface W . T —Ambient temperature.

§ 4.1.1. *Criticism of physical theory.*—This purely physical theory fails to account for the exudation pressure exhibited across a cut surface of a stem, also the guttation of water from leaf tips when the plants are kept in an atmosphere saturated with water vapour. For explaining these two observed facts it is necessary to postulate activated transport through the root cells as well as probably through the living cells lining the xylem vessels. The claim of the theory is based upon its apparent ability to account for rapid evaporation from leaf surface. Here there is another set of observations which shows that under certain conditions transpiration current can flow from leaf to stem. Further it has been observed that many water-containing elements of the xylem are filled with gases during summer or during periods of water deficiency and are filled up again during winter or during conditions of available water supply.

We shall in a subsequent section (§ 5) discuss the channel of translocation of organic food material manufactured in the leaf system to the stem and the roots, as well as the mechanism of translocation. It is generally accepted that such movement of food materials takes place through the sieve tubes of the phloem, which contain cytoplasm but no nuclei. We have described earlier a group of investigations of which the most recent ones are by E. S. Bollard (1953, 1956), who reports, from chemical and chromatographic examination of tracheal saps of a number of dicotyledonous, monocotyledonous and gymnosperm trees, as well as of some herbaceous plants, that amongst the nitrogenous compounds only a trace occurs as inorganic nitrate, while over 90 per cent occur in the form of amino-acids and amides as well as some peptides, besides organic phosphates.

It appears that there is not much qualitative difference in the nature of the food materials translocated through the stem from the leaf system as well as from the root system. From the above discussion two important observations stand out:

(i) The experiments of Hoagland and others who have found that when nutrients containing radioactive isotopes are applied to the roots, they appear to travel preferentially through the xylem vessels of the stems rather than through the neighbouring living cells to phloem as well as through the parenchymous cells,

(ii) the mechanism of translocation of root sap to the rapidly transpiring leaf system during period of high evaporation appears to be better interpretable by Dixon and Joly's hypothesis of imbibition pull transmitted from the leaf to the root through an unbroken liquid column whose cohesive force can withstand great tensile pull.

§ 4.2. *Vital theory.*—The advocates of this type of theory maintain that while some physical forces play undoubtedly some part in the process, but at the same time continuous ascent of water in the xylem is possible only in the presence of living cells. The exact functions, however, that these living cells perform are not clearly set forth. Bose (1923, 1928) and later Molisch (1928) attribute the ascent of water practically entirely to the physiological activity of the living cells of plants. Since the works of Bose had at the time of their publications attracted much attention, Miller in his book *Plant Physiology* (1938) has given in considerable detail account of Bose's theory, as well as some criticisms of the methods used by Bose and of the conclusions that he had deduced from his experimental evidences. The following account is based upon a monograph by the writer entitled 'J. C. Bose's Plant Physiological Investigations in Relation to Modern Biological Knowledge' (1947-48).

§ 4.2.1. *J. C. Bose's theory.*—The aim of these investigations of J. C. Bose was to show that sap is propelled in plant tissues from the root to the leaf mainly by the pulsatory activity of a layer of plant cells lying in a special region, the inner cortex; the process is more physiological than physical, as proposed in the theories of Dixon, Joly and Askenasy.

The theory of cellular pulsation asserts, that (1) the ascent of sap is due to the independent activity of living cells which extend throughout the length of the plant, and hence, neither transpiration nor root pressure is essential to the process; (2) propulsion is due to cellular activity, which is enhanced under increased internal pressure, consequently the rate of ascent should diminish under condition of drought, and (3) it is not the dead xylem vessels but the living cells which take active part in the conduction, hence a previous exposure to air of a cut stem should not cause a subsequent reduction in movement of sap through it.

The experimental investigations carried out by Bose can be divided into two groups:

(i) Those which relate to the different conditions under which sap is conducted through a plant stem; and (ii) those which relate to the finding of the particular channel in the stem which takes an active part in the conduction and the mechanism of such conduction.

Results.—(i) It was shown that the movement of sap takes place in stems from which both leaves and roots have been removed and that all the factors, e.g. like heat and cold, the effects of stimulants and depressants which affect the other physiological activities of the plant, like response to stimulation, transmission of excitation, etc., also affect in a similar way the conduction of sap in the plant stem.

(ii) It was shown that there is a definite layer of tissue, viz. in dicotyledonous plants, the innermost layer of the cortex adjoining the xylem which is responsible for sap conduction. This active cortex abuts the

phloem, which has been found to be the channel of conduction of excitation in *Mimosa* plant. The phloem is separated from the young xylem, the alburnum by the cambium layer. The sap is propelled by active pulsation of alternate contraction and expansion of the cells in the active layer, which forces the water not only upwards in the physiological conduction of sap, but also in a lateral direction into the contiguous alburnum. The latter is the channel for the mechanical transport of water, the force of injection is supplied by the active cortex. The bulk of the xylem serves as reservoir, the water being pumped into or withdrawn from it according to circumstance.

An accepted method of distinguishing whether any transfer process in a living organism is due to purely physical forces or is dependent upon utilizations of chemical energy, is to measure its rate of increase with temperature. In general the temperature coefficient of diffusion, referred to a temperature interval of 10°C. (Q_{10}), has been found to be 1.2 to 1.3 while the temperature coefficient of chemical reaction varies between 2 and 3. Bose found that the rate of sap conduction was double for a rise of 5°C. of temperature. The observed rate of temperature dependence is in agreement with the theory that the ascent of sap depends upon plant metabolism.

Use of electric probe and of sphygmograph.—It has been shown that by the introduction of an electric probe, it is possible to find out a layer whose cells are in a state of electrical activity due to the transmission of excitation through it. This method has been previously used by Bose to find out the specialized tissue in the petiole of *Mimosa* through which nervous excitation is transmitted. Assuming that the sap in the plant stem is transmitted by the pulsatory activity of a particular layer of cells, it would be possible to detect the depth of this layer from the stem surface by means of the electric probe. The pulsations are due to a rhythmic loss and gain in turgor, and should be accompanied by reversible changes in the electric polarization of the cellular membrane. It was found that during the ascent of sap through a plant stem, the inner cortex gave rise to electric pulsations. Further it was found that conditions which stimulate or depress these electric pulsations cause parallel modifications in the rate of ascent of sap through the stem. Hence it was concluded that this pulsating layer represents the propulsive tissue through which the sap is transmitted. The electric pulsations of the propulsive tissue should be accompanied by pulsating volume changes in the stem, which can be measured by a high magnification sphygmograph. Bose claims to have found that any agent which enhances the pumping activity causes an increase of sap pressure indicated by an ascending curve, the expansive up stroke of the constituent pulsation being larger than the contractile down stroke. Conversely depressants diminish the pressure, as shown by the descending curve, the down stroke of each individual beat being larger than the up stroke. This rhythmic propulsion of sap along the inner cortical tissue in plants appears to J. C. Bose to bear a great deal of similarity with peristalsis by which the contents of the alimentary canal are carried along it in animals.

Where transpiration is feeble, the normal ascent along the cortex supplies every portion of the tree with water, the leaves become turgid and the xylem is filled with sap. During active transpiration, the physiological conduction is not sufficient to meet the demand and water is withdrawn from the xylem reservoir. Two factors are brought into operation, physiological conduction by and along the cortex, and physical transference along the xylem.

§ 4.2.1.1. *Criticism of J. C. Bose's theory.*—Dixon and his co-workers repeated certain phases of J. C. Bose's work, but were unable to verify his results (Miller, 1938). There have been criticisms also on other grounds. One of the chief criticisms is that the relationship reported by Bose and Molisch of the pulsations or rhythmic activity ascent of sap has not been definitely established. It does not follow that because pulsation activities and ascent of sap respond in a like manner to the same factors, that one is the cause of the other.

Benedict (Miller, 1938) has measured the cross-sectional areas of living cells in the tree trunks of 10 different species and computed the maximum pumping capacity of the most rapid pulsating rates found by Bose. The actual rate of flow under the maximal condition of transpiration proved to be 8,000 to 30,000 times as rapid as would be possible under the theory proposed by Bose. It has further been subsequently found sufficient to account for the rapid movement of solutes through the root cells as due mainly to activated transport, and no observations have so far been recorded of pulsating activity in the root cells (§ 7 concluding portion).

§ 5. *Rôle of the phloem in the transport of nutrients in plants.*—Before we can consider the possibility of a unified physiological theory of nutrient transport mechanism in plants, it is necessary to discuss our present state of knowledge of the mechanism by which food materials, principally produced in the leaf by photosynthesis, are transported to different parts of the plant, where they are utilized for metabolism, for growth, or are deposited as insoluble food materials like starch and proteins in storage organs. The channel for such transport is the phloem, with its sieve tubes containing cytoplasm but no nuclei. The sieve tubes are closed by perforated end plates, through which protoplasmic continuity is maintained between all the living cells, from the leaf to the roots, to the apices of the growing shoots, and to the various places of storage in the living plant. This is the equivalent of symplast theory of Munch. Through the phloem are transported the soluble organic nutrients, the sugars, the amino acids, the organic phosphates. During spring the direction of transport of food material is reversed. Starch and proteins are hydrolysed to yield simple soluble molecules which now move through the phloem sieve tubes often in the opposite direction to the growing regions of the plant.

Under certain conditions the salts which are secreted into the xylem vessels from the roots and are carried by mass transport to all the transpiring parts of the plant, when the cells adjoining the xylem vessels take up the solute, absorb them or conduct them through the rays to the bark. The portion of the salt not used up for growth nor accumulated in the tissues are carried down by the sieve tubes to the roots, and may be returned to the surrounding medium. There are some evidences that radioactive phosphorus introduced as potassium phosphate makes a complete cycle from root to root in three to six hours. Such radioactive phosphorus when injected into the phloem can travel both upwards as well as downwards in the sieve tubes of the stem.

§ 6. *Unitary mechanism for nutrient transport.*—There seems thus to be a large number of observations accumulating, which point to the existence of unitary mechanism by which nutrients, absorbed by the roots from the surrounding medium and partially converted into soluble organic molecules in the root cells, as well as the simple soluble organic molecules manufactured in the leaf, are circulated through the whole plant extending from the root to the leaf. Barring the dead xylem vessels the path taken by the solutes together with the solvent water is through the living cytoplasm, either as protoplast enclosed within semipermeable outer and inner

(tonoplast) walls, or as symplast inter-connecting all cells from the leaf to root. We may recall here that in Dixon and Joly's theory of transport of water through plants, the water in the conductivity tract of the xylem exists in the form of an unbroken column, and these columns are continuous both vertically as well as horizontally through the cell walls, i.e. a continuous meshwork of water exists throughout the whole length of the stem, root, and leaves whose terminals are, in the opinion of these authors, the menisci of water in the submicroscopic cavities of the walls of the epidermal cells in the root as well as in the leaf. Since it is recognized that the transport of water is essentially a physiological process (e.g. in sap exudation from leafless cut stem and leaf guttation in humid atmosphere) there will be no great theoretical objection if we identify this unbroken water column of Dixon and Joly with the symplast of Munch which has lateral connections with the xylem vessels. With this assumption we shall discuss the various mechanisms which have been proposed to account for the transport of solute and solvents through cell cytoplasm and symplast, including the phloem sieve tubes.

The different mechanisms proposed for this transport are either one or combinations of more than one of the following processes.

§ 6.1. Diffusion along a concentration gradient, which may be either along protoplasmic thread of the symplast or across a membrane separating the protoplast of a cell from an adjoining medium, which can be xylem vessels, other cell protoplast or portion of the symplast, or the tonoplast separating the protoplasts of cells from the enclosed vacuoles.

In many instances transport through the sieve tubes take place from places where the nutrients are produced, e.g. in the leaf parenchymous cells, to places where such solutes are utilized for growth or metabolism of cells or are stored. Hence there is a movement along a concentration gradient but the physical diffusion process appears too slow to account for the observed rate of transport. A phrase, activated diffusion, has been coined to denote this process, but its exact mechanism is not known.

§ 6.2. There is an alternate theory of mass transport, in which both solute and solvent move together through the sieve tubes, due to difference in osmotic pressure caused by the concentration gradients of each solute component in the sap. The model of such mass flow is taken from the movement of sap in xylem vessels due to imbibitional forces. As examples of mass flow through sieve tubes is cited the bleeding of palm trees like *Cocos* and *Arenga* (Arisz, 1952). The cause of bleeding is situated in a more or less small area stimulated by bruising some time before the bleeding starts. Such exudation has been observed in *Arenga* for a period of 50 days during which 28 kg. of sucrose in 220 litres of sap were transported. This is an abnormal process, during which the stored starch of the stem is used up. It is not clear what happens to the aqueous portion of the sap, from which the nutrients have been absorbed either in the growing or storage regions of the plant. In the case of xylem transpiration the excess of water moved is lost by evaporation or guttation from the leaves. Does this aqueous portion of phloem sap return to the xylem vessels?

§ 6.2.1. *Transport by means of cytoplasmic streaming.*—Investigators like Goldacre (1952) attribute the accumulation of material against concentration gradient in cytoplasm to the phenomena of folding and unfolding of protein molecules. It is generally assumed that rhythmicity is one of the attributes of living cytoplasm; the myosin and cardiac muscles are specialized forms of cellular organization, in which rhythmicity is predominant. From this assumption it follows that the protein molecules forming the cytoplasm framework can rhythmically unfold and fold; the

energy required being mediated by ATP activity. When protein molecules unfold more surface areas become available for absorption of other substances, than when they are folded up. The side chains and other groups which were used to hold one part of the peptide chain to another part in the folded globular protein molecule, become free and are exposed to the solution and can absorb other substances.

Movement in the ameba which is due to the formation of pseudopodia in the direction of movement and the retraction of the cytoplasm in the rear can be cited as an example of protoplasmic folding and unfolding. The movement is a consequence of folding up of the protein molecules by which they reversibly change from gel to sol condition. The liquefied folded protein molecules stream forward to form pseudopodia. In the unfolded state the protein molecules in the cortical and plasma membrane can absorb materials from the surrounding, which they desorb when they fold up. Thus it has been found that ameba placed in dilute neutral red solution (0.003 per cent) accumulate the dye in their tails, only when streaming.

In many plant cells streaming often occurs in many directions at once, and has a complexity so bewildering that it is difficult to discern what is happening. The root hair of plants, which are single elongated cells, has a simplicity of structure comparable to that of the ameba; it is much narrower than the ameba. A typical one has a vacuole near the top and it is observed that the cytoplasm streams back and forward over the vacuole reversing bodily over, every five minutes. On immersing the cell in a neutral red solution, the dye accumulates in the vacuole, at the end toward which the cytoplasm was streaming. Goldacre (1952) states that this accumulation of dye is due to unfolding and folding of cytoplasmic membrane surrounding the vacuole. Some investigators like Curtis and Clark (1950) attribute the mass flow through phloem sieve tubes to such cytoplasmic streaming. The folding and unfolding of protein molecules which is the basis of such cytoplasmic streaming is an activated process mediated through ATP. How the absorbed material is transferred from one rhythmically pulsating cytoplasmic unit in the phloem tube to the one in front of it has not been explicitly discussed. Other investigators have concentrated their attention to the rôle of mitochondria particles which are carried to and fro along the cytoplasmic flow as the vehicle for transport of nutrients.

§ 6.2.2. *Rôle of mitochondria.*—During recent years intensive studies have been made on a group of particulate bodies the mitochondria whose presence in plant and animal cells has been established by staining technique. D. P. Hackett (1955) has given an exhaustive and valuable review entitled 'Recent Studies of Plant Mitochondria'. The following account is based upon Hackett's review.

The term mitochondria refers to the collection of variously shaped particles usually 0.5μ to 1.0μ in diameter, and up to 10μ or more in length, which are composed largely of lipids and proteins (in addition to water) and generally stain with Janus Green B. Recent biochemical studies have established these particles as the centres of a variety of metabolic reactions. In biochemical terms, 'mitochondria' can be defined as 'cellular particles associated with enzymes of the cytochrome system, the Krebs's cycle, fatty acid oxidation, and oxidative phosphorylation. Another group of still smaller particles 20 to 40 $m\mu$ in diameter, have recently been identified in plant cells and are referred to as microsomes; whether they belong to a separate class with special staining and biochemical characteristics has

not been definitely established. For the present discussion they will be included under the general term 'mitochondria'.

These mitochondria bodies are seen under the microscope to be subject to Brownian movement superposed on a general bodily motion in the cytoplasmic streaming. These particles appear to cluster in regions of high metabolic activities; inside the cells for example, along the outer boundary of the protoplast, round the vacuole, the cell nucleus, etc.

Hackett has enumerated the rôle of mitochondria in plant physiological processes as follows:

Any energy requiring process within the cell will be directly or indirectly dependent on mitochondrial metabolism. This is illustrated by the wide variety of inhibitory effects resulting from treatment of intact tissues with DNP which interferes with phosphorylation in the mitochondria, given as follows:

Inhibition of protoplasmic streaming, growth, glucose absorption, water uptake, salt accumulation, cell division, and tissue differentiation. Further any physiological activity which is linked with the functioning of the Krebs's cycle or the cytochrome system may be said to involve the mitochondria. Apart from these indirect connections there is growing evidence that the mitochondria themselves are directly involved as the instrument by which certain physiological functions are exercised. Most of the evidence along with these lines come from studies on animal mitochondria. Hackett gives some examples taken from the latter field in the belief that it represents a fruitful field for future botanical research.

The transport of water in living systems is closely geared to respiratory activity and to energy yielding reactions; it has been also suggested that non-osmotic or active mechanism may be involved. Such a possibility has frequently been considered for plant cells with reference to the mechanism of auxin action. Evidence on this is not definite but, as Hackett remarks, 'If there is a cytoplasmic water pumping mechanism it may well be located in the mitochondria'. Attention had been drawn earlier to the fact that mitochondria both within the cell and when isolated behave much like osmometers, and that a supply of energy is required for their morphological integrity. The swelling of mitochondria in hypotonic solutions can be reversed by ATP. We have discussed earlier (§ 3.2), while considering the mechanism of cellular salt transport, the rôle of phosphorylation and the activity of the cytochrome oxidize system, both of which are now found to be associated with mitochondria bodies. Sutcliffe (1953) has suggested that mitochondria may act as carriers in transporting ions across the cytoplasm to the vacuole. Radioactive potassium and phosphate have been found to accumulate in cytoplasmic granules of *Nitella*. Marked accumulation of radioactive calcium or phosphate ions have been found in mitochondria and microsomes of fractioned potato tuber slices, which have been kept in contact with solutions containing these ions for 24 hours. Work with isolated animal mitochondria have shown that the concentration of inorganic ions such as sodium or potassium can be greater in these bodies than in the surrounding medium and requires active metabolism for maintenance of the gradient. Likewise there can be an intramitochondrial accumulation of organic acids, such as ketoglutarate, fumarate, succinate, pyruvate. It thus appears that probably all the activated transport of both inorganic as well as organic compounds in the intracellular level is mediated through mitochondria system. We can picture them as shuttling between the different regions of active metabolism in cell protoplast and thus taking part in the transport process.

§ 6.2.2.1. All such problems of transport of organic and inorganic solutes into root cells, the transport through the protoplast to the vacuoles, the production of higher salt concentration compared to that in the outer medium in the protoplast, in the vacuole, as well as in the root xylem vessels are, we see, associated with the active transport movement mediated by the mitochondria. It appears that the displacement of mitochondria due to translation with the protoplasmic streaming, on which is superposed random Brownian movement of the mitochondria particles, may play an essential part in the mechanism of solute transport from the root cells to root xylem vessels. Lundegardh (1950) assumes that there is an active transport of solute molecules across the semipermeable membrane of the endodermal cells bordering the root xylem vessels, and a redox mechanism is also active there.

§ 6.3. *Mass transportation in phloem tubes.*—The next problem is the mechanism of transport of solutes in the symplast, which is supposed to be of the nature of active diffusion of an unknown nature, postulated by investigators like Curtis and Clark (1950). Is this in any way related to transport by mitochondria bodies as it occurs intracellularly? There is an indication of such a possibility in an investigation reported by J. H. McClendon (1952) of the occurrence of a high concentration of mitochondria in the phloem cells of tobacco leaves, suggesting a possible rôle in transport. Since the translatory motion of the mitochondria particle is much greater than what may be possible due to purely physical diffusion, the process of active diffusion due to transport by mitochondria-like bodies within the symplast can be understandable. It is not quite clear what factors control the directions of the mitochondria transport, so that the direction of transport of solute molecules may get reversed during different seasons of the year. All that can be said at present is that the transport by mitochondria particles is a process dependent upon substrate metabolism and as such is an active transport process; some degree of observability is attached to it. Since it is generally accepted that sap transport through the phloem is an activated process, there is no incompatibility in assuming that the mitochondria bodies are associated with it. What is not known at present is the exact mechanism of protoplasmic streaming by which the mitochondria are moved and how it is controlled by mitochondrial activities.

§ 7. *Velocity of transport through phloem and xylem.*—Use of labelled atoms to determine the velocity of transport of nutrients through xylem vessels and phloem sieve tubes has given some surprising results.

Biddulph and Markle (1944) find the movement of phosphorus in phloem to be of the rate of 21 cm./hr. or even more. The authors consider such rapid movement is not explicable even on the basis of protoplasmic streaming. Kursanov (1956) in his report gives even more striking values. It is mentioned in his report that speed of transport of products of photosynthesis from leaf to root system is of the order 70–100 cm./hr. while that of ascending sap may be about 180 cm./hr. In some of the trees the rate of water movement can be of the order of 6–8 metres/hr. Whether such rapid rate of movement in phloem tubes can be explainable by the utilization of mitochondria movement remains to be determined.

Another set of observations (Hoagland, 1944) shows that labelled phosphorus and other radioactive atoms applied to the roots appear much earlier in the xylem sap than in the bark. This requires to be accounted for in any physiological theory of sap transport.

§ 7.1. *Interpretation of high velocity nutrient transport.*—The following is a tentative explanation of such observations. The salts absorbed

from the soil by the root cells can travel along two alternate paths. One by transference from cell to cell from the epidermal layer of the root cells to the endodermal cells adjoining the xylem vessels. The driving pressure is generated by the redoxpotentials at the cellular interfaces from epidermal to endodermal cells. The sap is secreted into the vessels under a pressure generated in the root cells, and since the diameter of the xylem vessels is comparatively large and the root pressure is of the order of several atmospheres the sap in the xylem can move upward impelled by root pressure only. In the flowering plants and young trees on which such experiments are carried out, the stems have lengths of the order of a couple of metres, along which root pressures can easily propel the sap along the xylem vessels. The other channel of nutrient movement from the root is along the symplast which connects the parenchymous cells. It consists of a system of tortuous narrow protoplasmic filaments connecting the root cells to the stem and leaf of cells. The resistance to sap movement along such narrow and tortuous channels may be comparatively large. This may lead to the observed rates of movements of 20 to 100 cm. per hour in phloem tubes, to 600-800 cm. per hour in xylem vessels. The rôle of the xylem vessel as suggested by J. C. Bose is to act as reservoir from which water may be either withdrawn or transferred from the adjoining plant cells according to the needs of the plant as a whole. The pulsating machinery does not exist in the pulsating cell as a whole, as pictured by J. C. Bose (1923), but in the rhythmically pulsating protein fabric of the cytoplasm, maintained by substrate oxidation (ATP phosphorylation). The mitochondria carriers of solutes are transported along protoplasmic streaming. The source of energy for such transport is oxidation phosphorylation.

SUMMARY AND DISCUSSION

In this article the present state of our knowledge of the mechanism underlying the translocation of nutrients in green plants has been reviewed.

The two principal sources of nutrient supply to the plants are the root system drawing nutrient mainly from the surrounding medium and the mesophyll cells of the leaf, which are the seat of photosynthesis. The nutrients absorbed or manufactured by these plant organs are circulated through all the living cells in the plant. The nutrients absorbed by the root epidermal cells are mainly inorganic in nature and after passing through the intervening root cells enter into the dead xylem vessels of the root stem; from there the xylem sap reaches all the individual living cells of the plant. Similarly the organic nutrients, manufactured in the leaf, circulate through another set of tube-like cells, the phloem sieve tubes.

For a proper functioning of the two sources of nutrient supply, it is necessary that the products of each source should be available to the other. The mesophyll cell of the leaves, which absorbs CO_2 mainly from the air, receives water and inorganic salts from the roots, while the roots require the soluble carbohydrates and other organic nutrients from the leaf.

In the present review attention is mainly concentrated on the mechanism of absorption of materials by the roots from the surrounding medium, how they are transported, sometimes after chemical modifications in the root cells, to the xylem vessels and finally how these nutrients are absorbed in the different plant cells.

In the case of mesophyll leaf cells, the process of manufacture of organic nutrients has not been discussed, but only the mechanism of transport through the phloem sieve tubes to the rest of the plants.

The topics dealt with in this article mainly are:

(1) Mechanism of absorption of nutrients from the surrounding medium by the epidermal root cells and their transport intercellularly from the epidermal to the endodermal root cells and finally to the dead xylem vessels.

Experimental observations show (a) that the concentration of many salts in the vacuoles of root cells is several times higher than that in the surrounding medium, (b) that the absorbed sap is transferred to the xylem vessel under a pressure gradient between the root endodermal cells and the xylem vessels, whose measure is the root pressure in the xylem vessels.

(2) The xylem sap ultimately reaches the transpiring top leaf cells of trees which can reach a height up to 200 ft. or more from the ground. The nature of the forces effective in raising large amounts of water to such heights in the trees has been subject of controversy for many years. The effective forces can be either physical or physiological in nature. It is generally admitted that both the factors are present, but the disputes are about the relative importance of these factors in particular, in cases of low respectively rapid transpiration.

(3) The last problem considered is the mechanism of transport through the phloem sieve tubes of the organic nutrients manufactured in the mesophyll leaf cells.

A large volume of experimental observations, probably not all of equal degree of reliability, has accumulated; these have been interpreted from different and often contradictory stand-points. One school of interpreters lays special stress on the purely physical factors like diffusion along concentration gradient, semipermeability of the cell membrane, which in special circumstances can lead to a state of Donnan equilibrium, where the concentration of an ion may be different on the two sides of a membrane freely permeable to the ion.

The movement of water in xylem vessels against gravity is explained by the evaporation from leaf cell menisci, in which the force of imbibition and tensile strength of liquid columns play their rôle. Here the work against gravitational force is attributed to latent heat of evaporation of water from the leaf surface. The rôle of physiological forces are recognized but are assumed to play a minor part.

Another set of investigators stresses the primary importance of physiological factors in effecting the transport of nutrients through the plant tissues, in which energy, released during substrate metabolism, is used up (i) during absorption of nutrients (in ionic form mainly) by the epidermal root cells, and during transport through the cell protoplast against solute concentration gradient, (ii) their transference through contiguous root cells to the root xylem vessels, (iii) the conduction of the root sap from the xylem vessels to living cells in the stem and the leaf system, (iv) similarly the translocation of the organic nutrients manufactured in the mesophyll leaf cells through the phloem sieve tubes to the living cells in the stem and root system of the plant.

In the present review an attempt has been made to present a unified interpretation of the significant experimental observations from the physiological stand-point. The basic observations and their theoretical interpretation relate to what occurs in the root system. These are absorption of nutrients from the surrounding medium by the epidermal root cells. Here besides the purely physical movement of solute *along* concentration gradient across a cell membrane, chemical forces are brought into action for effecting solute transport *against* concentration gradient.

Lundegardh's theory of anion transport through epidermal root cells, in which cytochrome and cytochrome oxidase system are involved, has been verified semi-quantitatively and supported by other observational data. It is now believed that Lundegardh's theory of anion absorption forms a part only of a more general theory which involves both phosphorylation of a carbohydrate substrate as well as the activity of the cytochrome and cytochrome oxidase (redox) system. A large volume of experimental observations reveals the central importance of the multiple enzyme system located in a group of particulate bodies present in cytoplasm and known as mitochondria; these bodies are carriers of both the redox system as well as of the phosphorylating enzymes. It is thus believed that mitochondria play an important rôle in the transport of solutes through the protoplast to the vacuole or to the membrane separating one root cell from its neighbour. The mitochondria bodies are seen to move about along the cytoplasmic streaming and the congregate at sites of intense metabolic activities, like the outer and inner protoplast (tonoplast) membranes as well as that round the cell nucleus.

Some investigators like Goldacre and Lorch ascribe the transport of solutes across the cell membrane through the protoplast to the rhythmic contractility of the protein framework of the cytoplasm. In the folded globular form the protein is in a sol state. In the unfolded state some of the peptide bonds which hold the molecules together are set free and can form attachments with materials surrounding it. This is the gel state of the protein molecule. When the protein molecule again refolds the attached materials are set free inside the cytoplasm. The energy of folding and unfolding of the protein framework is mediated through the ATP activity and ultimately by the substrate breakdown brought about partly by the mitochondria particles. This folding and unfolding of the protein framework of the cytoplasm leads to the observed streaming in the cytoplasm. At the present stage of our knowledge it is not possible to apportion in the transport mechanism the rôle of protoplasmic streaming as well as of the mitochondria.

It is assumed here that the mitochondria, carried along with the cytoplasm streaming, forms the unit responsible not only for the transport of solutes intracellularly but through the phloem sieve tubes as well. The presence of mitochondria has been reported in phloem tubes of some plants.

Munch's theory of a symplast by which the cellular cytoplasm of all plant cells are connected with each other by means of cytoplasmic filaments can be made the basis of a theory of transport of nutrients both arising in the root system as well as that manufactured in the leaf system through the common symplast. In this hypothesis the rôle of the xylem vessels is that for storage of water with its solutes. These vessels have access to living cells surrounding them. According to the state of turgor of these border cells, water can be either taken up from the xylem vessel or released to them.

Details of this theory as well as a historical account for the previous theories have been included in this review.

REFERENCES

- Arisz, W. H. (1945, 1947⁴¹, 1948). *Proc. Acad. Sci. Amsterdam*, **49**, 420; **50**, 1019, 1235; **51**, 25.
 ——— (1952). Transport of Organic Compounds. *Am. Rev. Plant Physiology*, **3**, 109.
 Bose, J. C. (1923). *Physiology of Ascent of Sap*.
 ——— (1928). *Motor Mechanism in Plants*.

- Bose, D. M. (1947-48). J. C. Bose's Plant Physiological Investigations in Light of Modern Biological Knowledge. *Trans. Bose Res. Inst.*, 17, 17.
- Bollard, E. S. (1953). *Nature*, 171, 571.
- (1956). *Nature*, 178, 1189.
- Biddulph and Markle (1940). *Am. J. Bot.*, 39, 275.
- Broyer, T. C. (1950). *Plant Physiology*, 25, 367.
- Curtis, O. E., and Clark, D. G. (1950). *Introduction to Plant Physiology*.
- Dixon, H. H., and Joly, T. (1894). *Proc. Roy. Soc.*, 57, 3. (See Miller for other references).
- Goldacre, B. J. (1952). Protein Molecules as Basis of Osmotic Work. *Int. Rev. Cyt.*, 1, 134.
- Hackett, D. P. (1955). Recent Studies on Plant Mitochondria. *Int. Rev. Cyt.*, 4, 143.
- Hoagland, D. R. (1944). Lecture on the Inorganic Nutrition of Plants.
- Kursanov, A. L. (1956). *Sci. and Cult.*, 21, 515.
- Lundegardh, H., and Burstrom, H. (1933). *Biochem. Z.*, 261, 235.
- (1935). *Ibid.*, 277, 223.
- Lundegardh, H. (1950). *Physiol. Plant*, 3, 103.
- (1955). Mechanism of Absorption, Transport, Accumulation and Secretion of Ions. *Ann. Rev. Plant Physiol.*, 4, 1.
- Miller, E. C. (1938). *Plant Physiology*, Chap. XII, Translocation of Materials in Plants.
- Molisch, H. (1928). *Nature*, 122, 168.
- (1928). *Science*, 69, 217.
- McClendon, J. H. (1952). *Am. J. Bot.*, 39, 275.
- Munch, E. (1930). *Die Stoffbewegungen in der Pflanze*.
- Osterhout, W. J. V. (1936). *Bot. Rev.*, 2, 283.
- Overstreet, R., and Jacobson, L. (1952). Mechanism of Ion Absorption in Roots. *Ann. Rev. Plant Physiol.*, 3, 1952.
- Sinnot and Wilson (1955). *Botany—Principles and Problems*.
- Steward, F. C., and Street, H. E. (1947). *Ann. Rev. Biochem.*, 16, 471.
- Sutcliffe, J. R. (1953). Ion Secretion in Plants. *Int. Rev. Cyt.*, 2, 179.



NOTICE

The Journal of the Asiatic Society (Science & Letters) is a continuation of the following six types of periodicals published by the Society:

Asiatick Researches, I-XX, 1788-1839.

Journal of the Asiatic Society of Bengal, I-LXXIII, 1832-1904.

Proceedings of the Asiatic Society of Bengal, I-XL, 1865-1904.

Journal and Proceedings of the Asiatic Society of Bengal, New Series, I-XXX, 1905-1934.

Journal of the Royal Asiatic Society of Bengal (Science, Letters and Year-Book), Vols. I-XVI.

Journal of the Asiatic Society (Science, Letters and Year-Book), Vols. XVII-

Single numbers are charged for at the rate of One Rupee for each 16 pages or part of 16 pages text, and for each plate, map, table, etc., not included in the text. Postage extra.

Members of the Society receive the current numbers of the Journal free, by virtue of their membership, and, if ordering back issues direct from the Society, have a right to a discount of 40% on their prices.

Bonafide Book-Sellers are entitled to 25% discount.

The Asiatic Society publishes also:

Bibliotheca Indica, a series of Oriental books, since 1849.

Catalogues of Manuscripts.

Monographs.

Miscellaneous publications.

The Society published also Memoirs from 1905 to 1940, and Numismatic Supplements from 1919 to 1938.

Information about the publications and price lists are obtainable from the Asiatic Society or its Agents.

A number of complete volumes or loose numbers of the older periodicals of the Society are still available for sale. Reprints of some of the important articles are also available for sale.

Revised prices of loose numbers of the Journal.

All previous prices as printed on the various "Journals" published by the Royal Asiatic Society of Bengal are cancelled.

Old loose numbers are available for sale at special rates.

All old sterling equivalents are cancelled.

Obtainable from the Asiatic Society, No. 1, Park Street, Calcutta 16, or from the Society's Agents:-

MESSRS. LUZAC & Co., 46 Great Russell Street, London, W.C. 1.

MESSRS. THACKER, SPINK & Co. (1933) LD., 3 Esplanade East, Calcutta.

MESSRS. THE MOORE-COTTRELL SUBSCRIPTION AGENCIES, NEW YORK, U.S.A.

MESSRS. OXFORD BOOK & STATIONERY Co., Scindia House, New Delhi.

Residents of Europe and America should order from the local Agents.

When ordering direct from the Society the following rules should be observed:-

Orders should be addressed to the Asiatic Society, and not to any Official by name or title.

All Cheques, Money Orders, etc., should be made payable to the "Treasurer, Asiatic Society".

In India books are supplied by V.P.P.