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VIEWPOINTS IN BIOLOGY

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Edited by
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VIEWPOINTS IN BIOLOGY

I

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INTRODUCTION

Viewpoints in Biology will publish broadly based reviews of biological subjects which may be extensively illustrated. These reviews should not only summarize the state of the subject but also indicate the direction in which progress may be expected, and stress unsolved problems. While putting a cogent, well-argued point of view the authors will, however, not necessarily be asked to give exhaustive documentations of all the work in the subject. Considerations of the theoretical aspects of biology, criticisms of well-established methods and discussions of material from an evolutionary point of view will all be welcome. Descriptions of the practical details of methods will not be accepted unless they are involved in the consideration of a subject of general biological interest.

As far as possible the reviews should be readily understandable to other scientists as well as biologists. With increasing specialization within biology as well as the sundering of science into departments, it is more and more necessary that the problems facing one sort of scientist should be presented in a way which is understandable to others, so that if their interests are aroused they can bring to bear their own specializations on to the problems of another department of science.

Contributions will be printed in English but may be submitted in French or German.

Suggestions for contributions should, in the first place, be sent in outline form to one of the editors:

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A guide to authors and information on fees will be sent when a suggestion is acceptable.

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THE PHYSIOLOGY OF ROOTS

H. E. STREET

INTRODUCTION

TEXTBOOKS relating to any particular branch of science tend to follow a traditional pattern. The chapter headings of a new textbook dealing with the physiology of plants can usually be quite accurately and quite properly predicted from a study of the classical texts. It is, however, an equally well established tradition for the teacher to endeavour to present his subject in one way and to choose as collateral reading for the student a book which follows as contrasted a pattern as possible. Further, to test the student's progress the teacher seeks to frame questions which do not correspond to the sub-headings of either his lectures or the recommended textbook. These practices follow from the thesis that it is the ability to see interrelationships between different aspects of a subject which is the hallmark of understanding.

A heading similar to the one chosen for this chapter will not readily be found in any textbook. While it does discuss some work only recently published or still unpublished, its framework comes not from such new knowledge but from an attempt to bring together, from a number of aspects of botanical enquiry, information regarding the physiology of the roots of seed plants. By so doing, familiar information may gain new interest and significance. Further, such an approach, by its attempt to be comprehensive, high-lights some of the important gaps in our understanding of the physiology of higher plants.

The root system clearly serves to anchor the plant in the soil, frequently acts as a site for food storage and normally, in land plants, functions as the organ mainly responsible for the absorption of water and essential mineral salts. These physiological functions are reflected in the general form and microscopic structure of roots. Studies of size and form lead to studies of the rate, duration, localization and control of growth in the developing root. Studies of microscopic structure, by revealing the characteristic pattern of tissues and the wide range of different cells present, lead us to enquire into the factors controlling cell differentiation and into the separate contributions and physiological interrelationships of the individual cells and tissues to the vital activities of the whole root. Man can conserve or dissipate the fertility of the soil: the aerial environment of the plant is less within his control. Hence, every development in our understanding of the influence of the nutritive and other soil factors on root growth and function is potentially of importance in plant husbandry.

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THE ROOT AS AN ABSORBING SYSTEM

Absorption of Water

Appreciation of the great extent and rapidity of growth in root systems followed from work on the prairie plants of the U.S.A. by Weaver and Clements¹ at the Carnegie Institution of Washington, and from the work of Rogers² at East Malling, on the growth of fruit tree roots. Examples may serve to illustrate this point. A two-year-old plant of the grass, *Agropyron cristatum* has been reported³ to possess 315 miles of root, occupying a soil volume of 2 m by 1.2 m: a plant of winter rye, grown for 4 months in soil, was reported⁴ to possess 387 miles of roots corresponding to an average production of 3.1 miles of root per day. In this latter case it was calculated that the new root produced each day invaded sufficient soil to provide for the total water requirements of the plant. Where, as must often be the case, the water content of the soil is such that only very slow movement of soil water occurs, then the invasion of new soil by root growth appears to be critically important in maintaining the water balance of plants. The very

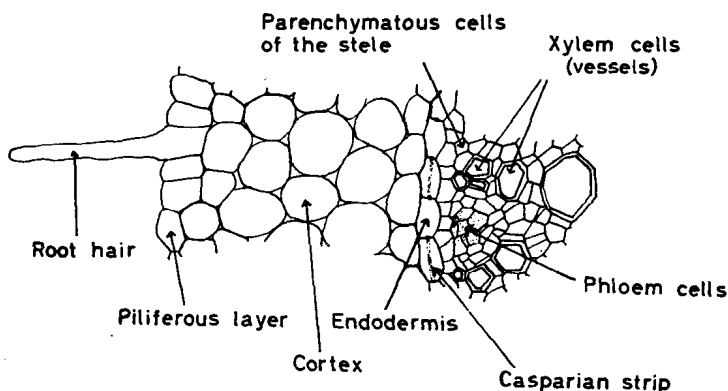


Figure 1.1. Diagrammatic transverse section of a root in the region of the root hairs

large quantities of water lost by plants on sunny days would require the root systems to have an enormous area of contact with the soil; for instance, a tree 10 m high may lose 200 l. of water per day by transpiration.

The uptake of water does not occur uniformly over the whole root surface. The most active region of water absorption is behind both the apical centre of cell division (the promeristem) and the region of most active cell elongation; it declines again as the outer layers of the root are rendered less permeable by the deposition of fatty material (suberin). This means that the most active region of water absorption from the soil is the region of root hair development, a region where the area of contact with soil particles is highest per unit length of root. The importance of this enhanced area of contact between soil particles and absorbing cells is a further indication of the slow movement of water in soils containing less than their maximum water content under conditions of free drainage (*i.e.* less than their field capacity). Studies of the development of root hairs in a moist atmosphere may give an exaggerated picture of the extent to which they enhance root

surface area in the soil. Nevertheless, root hairs on actively growing root systems in the soil probably increase root surface area by a factor of 2 to 5. We know that the functional life of root hairs is limited, that it may be as short as a few days. There is, however, little precise information regarding the natural variation in the duration of function of root hairs as between species and as affected by soil conditions. Nevertheless, it is clear that the role of root hair development in determining the absorbing capacity will only be of importance in actively growing roots. Rosene's work⁵ at the University of Texas indicates that in seedling roots, the root hairs have only a

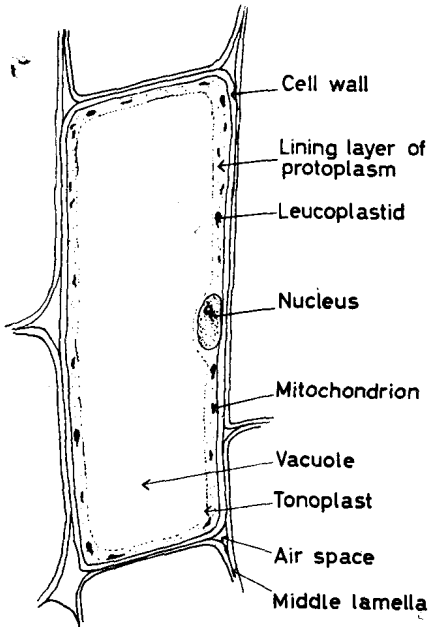


Figure 1.2. Diagrammatic section of a mature cortical cell of the root

similar permeability to water to that of other surface cells of the root. Examination of the roots of trees strongly indicates that water absorption by regions free of root hairs and even by suberized regions must, in many cases, account for the greater part of the water uptake. The mechanism of water uptake by suberized roots is at present obscure and in need of critical study.

The process of water uptake by the root involves not only the initial absorption but the distribution of water to the internal tissues. It is, therefore, necessary to consider the structure of the root—the nature and arrangement of cells in the root tissues. Figure 1.1 is a diagrammatic representation of the transverse section of a root in the region of the root hairs. The cells of the piliferous layer, including the root hair cells, those of the cortex, including its special innermost layer, the endodermis, the parenchymatous cells of the stele and the phloem cells are living units. The living contents (protoplasts) have in the piliferous layer and cortex a large central aqueous vacuole separated from the enclosing wall by a sheath of protoplasm (Figure 1.2). The xylem cells (vessels or tracheids) when fully mature are represented

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only by their cell walls. The root hair and piliferous layer cells have, like those of the parenchyma of cortex and stele, walls of cellulose and pectic substances which exert a strong attraction for water. The outer part of the walls of root hairs is slimy and strongly basophilic. This layer, particularly near the tip of the root hair, is permeated by a 'fuzz' of cellulose strands (as seen by electron microscopy) which develop intimate contact with soil particles. The living tissue of the cortex always has an intercellular air space system, the extent of which is influenced by the soil environment. This system only floods under conditions unfavourable to root growth, suggesting that the wall surfaces lining the air spaces are water repellent. The central stele, which functions in the longitudinal transport of water and solutes, is surrounded by a single continuous layer of cells constituting the endodermis. The radial and transverse walls of its cells, very early in their development, show evidence of the deposition within them of fatty material (possibly suberin). This constitutes the Casparian strip, first studied by Caspary in 1858 and readily revealed by its characteristic staining reactions. Fluorescein and other soluble materials capable of diffusing rapidly in the cell walls of the external cortex do not penetrate beyond this Casparian strip. Priestley¹¹ and others have stressed the importance, in the water and salt relations of roots, of the development of this Casparian strip on the grounds that it ensures that substances passing in either direction across the endodermis must pass via the living protoplasts of the cells.

It has long been known that the longitudinal movement of water in the root, and from the root into the shoot system takes place in the xylem cells, the vessels and tracheids. However, the state of this water in the xylem cells is dependent upon environmental conditions. Under conditions favourable to 'metabolic activity' but in which the rate of loss of water from the shoot by evaporation is very low, the water in the xylem cells is under positive pressure. This pressure can be demonstrated as a positive 'root pressure' by cutting off the shoot at ground level and attaching a manometer to the exposed transverse section of the root—an experiment performed by Evelyn in 1670 and by Hales in 1727. By contrast, under conditions of rapid water loss by evaporation from the shoot (*i.e.* with rapid transpiration) there develops a negative pressure in the xylem cells; the xylem water is under tension by virtue of a 'transpiration pull'. In the first case, the motive force of water movement is generated by the living root; in the second case, the water movement is a 'transpiration stream'.

The development of positive root pressure and the associated phenomenon of the 'guttation' of liquid drops of water from leaves are clearly the result of an 'active' absorption of water by the living root. Such root pressures frequently exceed 1 atm and White⁶ at the Rockefeller Institute, using tomato root cultures, has recorded a root pressure of 6 to 7 atm. These positive root pressures develop only when there is a favourable temperature and adequate aeration, and their magnitude is affected by the ionic strength of the soil or nutrient solution in which the plant is growing. This 'active' water absorption is not capable of extracting from soil more than half the water removable under conditions of active transpiration. It may be of importance in submerged aquatic plants as a means of generating a flow of water and dissolved substances in the xylem cells. Kramer⁷ of Duke

University has, however, argued strongly that it is of negligible importance in the over-all water relations of land plants, pointing out that 'active' absorption, under the most favourable conditions, only transports less than 5 per cent of the water moved by the actively transpiring plant and that positive root pressures can only rarely be demonstrated in conifers.

The mechanism leading to the generation of root pressure is a matter of dispute and to understand the problems involved it is necessary to refer back to the cellular structure of the root. There is strong evidence, from microscopy and from studying the penetration of dyes into cells, that a protoplasmic membrane (the tonoplast) is present at the surface of the cell vacuole. Whether a cell membrane also occurs at the protoplasm-cell wall junction is more difficult to determine, particularly as normally the protoplasm not only penetrates the cell wall as microscopically visible strands connecting the protoplasts of adjacent cells, but also may permeate the cellulose framework of the whole wall. Now ever since the classical researches of Hugo de Vries in 1871, we have known that the vacuolated plant cell can behave, under certain experimental conditions, as an osmotic system. The direction of, and force involved in water movement between the cell and its bathing solution depend upon the difference (called the suction force) between the osmotic attraction of the vacuolar solution tending to draw water into the cell and the sum of the external osmotic attraction of the bathing solution plus the inward pressure of the stretched cell wall (wall or turgor pressure), both of which tend to drive water out of the cell. When the water balance in the cell is determined by this relationship, the vacuole and the bathing solution lose or gain water, respectively, as if separated by a membrane permitting passage of water molecules but not of dissolved substances. Such a membrane was termed by Moritz Traube in 1867 a 'semipermeable' membrane. The operative cellular membrane is not the cellulose-pectin cell wall, which is readily permeable to solutes, but must be a protoplasmic membrane and, as indicated in the above description, it is generally considered to be the tonoplast membrane bounding the vacuole which confers upon the cell its osmotic behaviour.

On rather inadequate evidence, the view is widely held that, during 'active' absorption of water by the root, water passes from the soil or nutrient solution into the root hairs and piliferous layer cells and across the cortex by a gradient of inwardly increasing osmotic attraction (suction force). On this view, it is visualized that, by osmosis, water passes through the outer cell wall, the adjacent layer of protoplasm, into the vacuole, then from the vacuole via the protoplasm into the inner wall and so on through each of the cortical cell layers to the endodermis. However, studies^{8,9} on the transport of salts and fluorescent dyes have provided evidence that solutes can move across the root without entering the vacuoles of the cortical cells and it has been calculated¹⁰ that this 'apparent free space' in which solutes move freely significantly exceeds the cell wall volume in the wheat root. Water movement across the cortex may therefore occur along a pathway which includes the cell walls, films of water on the walls and the lining layers of protoplasm of the cells, but which does not involve penetration of the vacuolar membranes.

Priestley¹¹ and his co-workers first stressed the importance of the

endodermis in the development of ⁵ positive root pressure and this has been supported by many later studies. Certainly, solutes which readily migrate in cellulose cell walls do not pass the Casparian strip. Furthermore, the cell wall in the region of the Casparian strip seems to be in particularly intimate contact with the protoplasm of the endodermal cells. The endodermis, therefore, may be the layer at which water (and solute) movement comes under protoplasmic control. This view is not universally accepted for evidence has been obtained¹² of rapid movement of solutes across the endodermis even when, as in the older parts of the root, its protoplasts become enclosed in layers of suberin.

The fact that positive root pressure is only observed under conditions promoting the physiological activity of the living root clearly points to a protoplasmic control of the underlying 'active' water uptake, but it does not indicate what aspect of the metabolism of the cells is most directly linked to this process. Priestley put forward the hypothesis that osmotically active substances arise in the xylem cells during their maturation, when disintegration of their protoplasts occurs, and that the living endodermis acts as the effective semipermeable barrier between the osmotic attraction of the liquid in the xylem and the soil solution. Atkins¹³ visualized an osmotic movement of water right through the living tissues of the root dependent upon the difference in osmotic attraction of the soil solution and the xylem liquid, postulating a secretion of sugars into the xylem by the living cells of the stele. However, more recent work suggests that inorganic salts, liberated by the living cells of the stele, are mainly responsible for the osmotic attraction of the xylem liquid. The contention in these hypotheses, that root pressure is a consequence of a difference in osmotic attraction between the xylem liquid and the soil solution, has remained a widely accepted view and in support of this Kramer⁷, for instance, has demonstrated that root pressure changes extremely rapidly in response to any variation in the osmotic activity of the solution around the root, just as if the root were acting as a sensitive osmometer.

The classical hypothesis that osmosis is the force regulating the water content of plant cells has already been outlined. However, of recent years there have been reported a number of instances where the hydrostatic pressure established in cell vacuoles appeared to be in excess of that expected from osmotic considerations. This has led to the postulation of a 'non-osmotic' force in water uptake, dependent upon the continuous expenditure of energy by the cell. If the water balance of cells is, in fact, regulated entirely or even in part by such a 'pumping' force, then it could move water into or out of cells. This is the basis of the alternative concept that root pressure is not the reflection of an osmotic system but results from an active secretion of water into the xylem cells. However, experiments using isotopically labelled water (tritium-labelled and deuterium-labelled water) show that cells are very permeable to water. Therefore, it seems unlikely that a pumping or secretory mechanism could operate against so 'leaky' a membrane. Nevertheless, it is still necessary to decide whether the 'active' component in root pressure is a secretion into the xylem cells, of solutes or of water. Further, we have little knowledge of the mechanism by which cells exhibit such secretory activity.

This consideration of active water absorption by the root has been dictated by its physiological interest rather than because of its importance in the water relations of the whole plant. As indicated earlier, the water in the xylem cells is normally under negative rather than positive pressure due to the development in the leaves of a transpiration pull. Actively transpiring plants can lower the water content of the soil to a point where it has a water absorbing power of, on an average, 15 atm; 'active' root absorption ceases when this water absorbing power of the soil is of the order of only 1 to 2 atm. From studies of the rate of intake of water by root systems attached to a vacuum pump it has been shown that resistance to flow falls when the root is killed, indicating destruction of protoplasmic regulation of flow and that the rates of water absorption achieved under active transpiration would indicate tensions of 5 to 30 atm in the xylem cells. Such negative pressure in the xylem cells opposes the attractions of the root cell walls and protoplasts for water and causes a flow of water from the soil to the conducting xylem cells. The extent to which this will reduce the water content of the root cells will depend not only upon the magnitude of the tension in the water columns in the xylem but also upon the water content of the soil and the resistance to water flow offered by the root tissues. The main resistance to water loss by transpiration is at the conversion of liquid water into water vapour within the leaves. Withdrawal of water from the root cell protoplasts to the point where there is no positive hydrostatic pressure within the root cells (to the point where the cells are no longer turgid) will, therefore, presumably only occur when the water absorbing power exerted by the soil begins to reach the critical value averaging 15 atm. The rate of water uptake thus seems normally to be determined by the rate of transpiration, the root providing an ever-expanding but passive absorbing surface.

Absorption of Mineral Ions

Many early botanists assumed that because mineral salts occur in the soil solution they must be absorbed with the water. This question of what, if any, is the relationship in roots between the processes of water and salt absorption is still unresolved. As will be illustrated in the ensuing account, root cells can selectively accumulate mineral ions. In such accumulation there is no sweeping along of mineral ions in a flow of water. It has, however, been suggested that the increased rates of salt uptake frequently recorded during periods of high transpiration rate may arise by a direct flow of soil solution via the 'apparent free space' into the xylem cells. However, this would seem to ignore the evidence for a controlling function of the endodermis. Alternatively, the increased rates of salt uptake associated with periods of rapid water uptake could be due to prevention of local depletion of particular ions at the site of their active absorption or to enhanced secretion of salts into the xylem cells associated with the greater water movement.

The soil solution is normally extremely dilute when considered in relation to the mineral ion requirements of plants. Great interest therefore attaches to evidence that roots can absorb basic ions (cations) directly from colloidal particles. Two hypotheses have been advanced to explain this uptake from

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soil particles of cations such as calcium, potassium, and magnesium. The first stresses that roots constantly release carbon dioxide during their respiration, this in turn forms carbonic acid and leads to an exchange of hydrogen ions for cations. Acid production by soil micro-organisms could effect a similar release. The released cations would be absorbed along with bicarbonate or other acidic ions (anions). The second hypothesis postulates a 'contact exchange' of ions whenever the colloidal root surface makes contact with the soil colloids. This exchange proceeds without the ions ever appearing in the soil solution. As regards the source of essential anions such as phosphate, nitrate and sulphate, the general view seems to be that they are absorbed only from the soil solution and that the plant does not directly influence the release of these ions from the solid phase of the soil. A constant release of such anions from insoluble solids or from microbial activity may, therefore, be critical for plant growth. Further consideration of the soil-root relationship along these lines would take us away from our main theme into the many-sided problems of soil fertility.

Intensive studies of salt absorption by roots, grown under standard conditions and detached from the shoot, were undertaken during the 1930's by Hoagland¹⁴ and his associates in the Division of Plant Nutrition of the University of California (Berkeley) and by Lundegårdh¹⁵ and his associates at the Royal Swedish College of Agriculture. In these researches the influence of concentration upon the uptake of different ions was studied. Different ions were found to differ in their rates of uptake, ions with a low charge density being usually particularly rapidly absorbed. Cations modified the rate of uptake of other cations, anions the uptake of other anions. Changes in reaction of the external solution towards acidity or alkalinity were found to be indicative of unequal rates of absorption of cations and anions. All such observations emphasized that ion accumulation was a selective process and that it involved movement of ions into cells against concentration gradients, often resulting in the building up of high internal ion concentrations from very dilute solutions. Perhaps even more important, these researches, confirming parallel studies undertaken with the algae *Nitella* and *Valonia* and with storage tissues, emphasized the importance of the physiological activity of the cells in determining their ability to effect salt accumulation. Ionic balance within cells was maintained despite the unequal uptake of cations and anions, by the physiological production or depletion of organic acids. Respiration of the root cells was essential and the accumulation process ceased in the presence of substances which inhibited respiration or which 'uncoupled' this process from the chemical reactions which lead to the conservation of the released energy in a utilizable form. This relationship was of particular interest, for not only was respiration essential to salt accumulation but whenever accumulation was proceeding the rate of respiration was itself enhanced. This led Lundegårdh to postulate that the total respiration of cells is made up of two components, a 'ground' respiration independent of salt accumulation and a 'salt' respiration.

Experiments in which cells are allowed to take up radioactive ions and are then placed in a nutrient solution containing the normal ions have demonstrated that ion exchange between the cell and its bathing solution takes place more or less rapidly and continuously. Therefore, presumably not

only the initial accumulation process but also the maintenance of the excess internal concentrations of ions requires the expenditure of energy. Now one of the principal factors controlling respiration rate is the availability of energy-acceptor molecules, that is, of molecules capable of energy enrichment, such as adenosine diphosphate which can be energy-enriched by further phosphorylation. Presumably, salt accumulation, by consuming cellular reserves of utilizable energy, will generate such energy-acceptor molecules and hence stimulate respiration.

These researches, therefore, pose two fundamental and clearly intimately related questions regarding salt accumulation by plant cells:

(a) How exactly is the energy released by respiration used to transport ions against their concentration gradients?

(b) What determines the selective nature of the ion uptake mechanism, a selectivity which varies according to both the inherited potentialities and physiological state of the cells?

In a typical cortical cell of the root, the lining layer of protoplasm contains the single nucleus and numerous smaller living inclusions, mitochondria (*Figure 1.2*). Each of these bodies and the vacuole is enclosed in its own membrane. The outer surface of the protoplast is negatively charged but, as previously discussed, the extent to which it is bounded by a membrane is controversial. Because of this negative charge at the protoplast surface and on the colloidal particles of the protoplasm, it might be expected that the positively charged cations would penetrate the protoplasm of the cell more readily and to a greater extent than would negatively charged anions, and there is a considerable body of evidence that this is so. In this sense, ions may penetrate into cells by diffusion and the amounts so entering could be considerable if the penetrating ions were incorporated into various chemical compounds by physiological activity—nitrates into organic nitrogen compounds such as amino acids, phosphates into sugar esters, metal ions into combinations with protein, and so on. Active accumulation, that is, an accumulation which itself requires energy, would, however, be involved in the passage of cations into vacuoles and mitochondria, and in the case of anions would probably be concerned in their accumulation in the lining layer of protoplasm.

In order to explain such 'active' accumulation and its selectivity the idea has been advanced by many workers¹⁶ that membranes which are impermeable to free ions are penetrated by the ions chemically combined with or adsorbed on to 'carrier' molecules located at the membranes (*Figure 1.3*). These carrier-ion complexes would then liberate their ions on the cell or cellular inclusion side of the membrane much more rapidly than the free ions can diffuse back along the concentration gradient. Selectivity would be determined, at any given time, by the relative frequencies of different 'carriers' in the cell membranes and by their individual mobilities and chemical affinities. Cell membranes are rich in phosphatides and proteins and one obvious suggestion is that the 'carriers' might be specific enzyme proteins, forming unstable enzyme(=carrier)-substrate(=ion) complexes. This interpretation is emphasized in the general term 'permeases' for solute carriers adopted by Monod and his associates at the Pasteur Institute of Paris. Using as a basis the assumption that the carrier-ion complex is

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equivalent to the enzyme-substrate compound, kinetic analysis requires that the reciprocal of the velocity of absorption of an ion ($1/v$) should, when plotted graphically against the reciprocal of the ion concentration ($1/s$), give a straight line of which the ordinate intercept is at the reciprocal of the maximum rate of uptake ($1/V$) and the slope is given by K_s/V where K_s is the Michaelis constant (Figure 1.4). Epstein¹⁷ and his associates have studied the uptake of ions by detached barley roots from simple and mixed salt solutions and reported results consistent with the hypothesis, set out above, that ion uptake involves the formation and breakdown of ion-carrier complexes and indicative of the functioning of separate carriers for sodium, potassium, calcium, magnesium, sulphate and phosphate ions. In the latter case, separate carrier sites seem to be involved for uni- and bivalent phosphate ions.

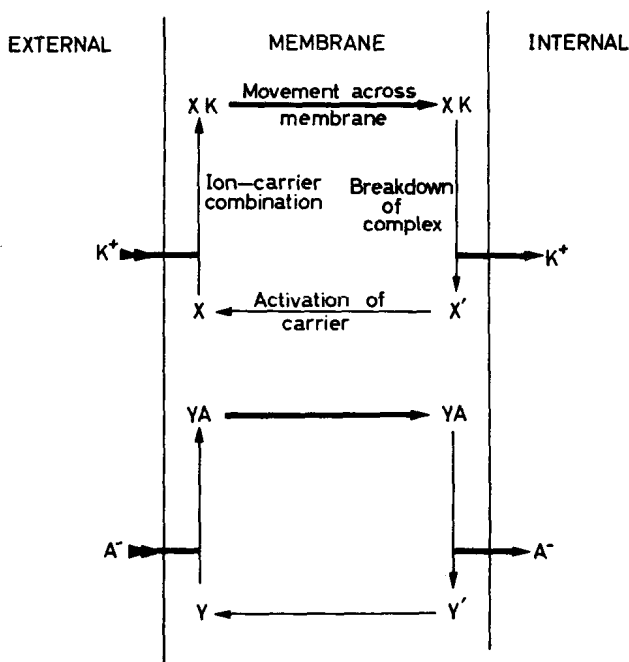


Figure 1.3. The general concept of the operation of a carrier mechanism in ion uptake by cells

X and Y = carriers. X' and Y' = precursors of the carriers.
 XK and AY = carrier-ion complexes. K^+ = cation, A^- = anion.

It must be emphasized that the isolation and identification of the postulated carriers has not been achieved but modern biochemical techniques should make such isolations possible in the near future. A brief outline of two interesting suggestions will, however, illustrate how the carrier mechanism could be energy-requiring. Bennet-Clark¹⁸ has suggested that the carrier could be a protein associated with the phosphatide, lecithin. The phosphate group in the phosphatide would act as a cation carrier, the basic choline group as an anion carrier. The liberation of the ions into the cell could be effected at the inner surface of the membrane by an enzymic

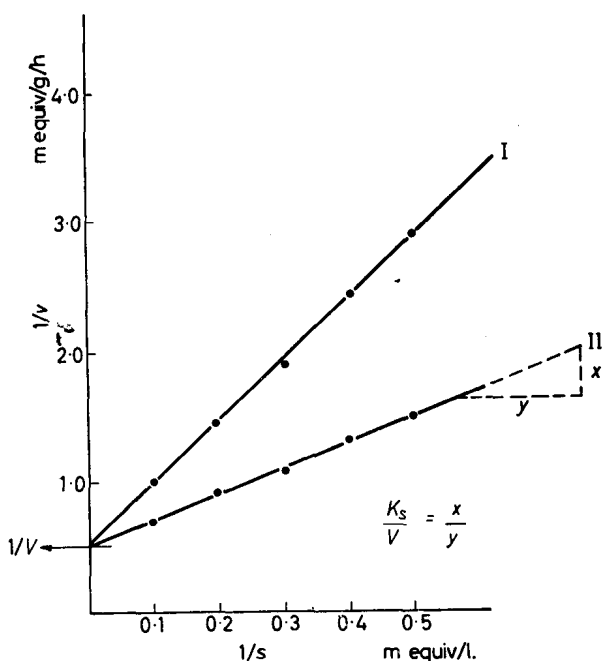


Figure 1.4. Plot of the reciprocal of ion concentration ($1/s$) against the reciprocal of the rate of uptake ($1/v$) for uptake of rubidium (I) from rubidium bromide and (II) from rubidium bromide in presence of potassium chloride (25 m equiv/l.). Conclusion can be drawn that Rb^+ and K^+ compete for the same carrier (= enzyme)

decomposition of the lecithin. Regeneration of the carrier following re-synthesis of lecithin would involve the utilization of respiratory energy. The second hypothesis was suggested by the properties of the contractile protein (myosin) of muscle. The molecules of this fibre-like protein can exist in both a contracted and an extended form. The extended form is energy-rich and may spontaneously contract; the unfolding of the contracted form consumes energy from the simultaneous enzymic decomposition of an energy-rich phosphate compound (adenosine triphosphate) formed during respiration. In 1952 Goldacre¹⁹ reported rhythmic movements of root hair vacuoles and suggested that they arose from ordered contraction and unfolding of protein molecules orientated in the vacuole membranes. Such a protein could, when its molecules are in the unfolded form, bind ions by free valencies exposed at the membrane surface. Contraction would draw these ions through the membrane and the act of contraction could lead to liberation of the ions as the free valencies of the protein became satisfied amongst themselves in the folded molecules. Energy, directly derived from respiration, could then unfold the protein and reset the trap (Figure 1.5). An attractive feature of this difficult-to-test hypothesis is that it clearly defines two states of the carrier, interconverted by use of a known energy source (adenosine triphosphate) and explains transport across the thickness of the membrane by making this a forceful displacement of the ions.